6-1-1973

The Use of Thin Layer Chromatography (TLC) in Qualitative and Quantitative Determinations of Photographic Developing Agents (in Developer Solution) Using a Scanning Densitometer

Michael Gilbert

THE USE OF THIN LAYER CHROMATOGRAPHY (TLC) IN QUALITATIVE AND QUANTITATIVE DETERMINATIONS OF PHOTOGRAPHIC DEVELOPING AGENTS (IN DEVELOPER SOLUTION) USING A SCANNING DENSITOMETER

by

Michael B. Gilbert

A thesis submitted in partial fulfillment
of the requirements for the degree of Bachelor of Science
in the School of Photography
in the College of Graphic Arts and Photography
of the Rochester Institute of Technology

June, 1973

Thesis Advisor: Dr. Ronald Francis

ACKNOWLEDGEMENTS

The author would like to thank Dr. Schumann, Dr. Francis, Professor Carroll and Professor Hill of the College of Graphic Arts and Photography, School of Photography at the Rochester Institute of Technology; Dr. Craven, Dr. Sowinski and Dr. Burns of the College of Science at the Rochester Institute of Technology; Elizabeth Solow, Assistant Professor, division of Neurological Surgery, Indiana University School of Medicine at Indianapolis; Dr. H. Baden of the Eastman Kodak Company, Photographic Technology Division at Rochester, New York; Richard Bilhartz and Barbara Roberts, my fiancee, for their assistance with the research and preparation of this thesis

Special acknowledgement is due to the C.I.A. for financial support for the research of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

THE USE OF THIN LAYER CHROMATOGRAPHY (TLC) IN QUALITATIVE AND QUANTITATIVE DETERMINATIONS OF PHOTOGRAPHIC DEVELOPING AGENTS (IN DEVELOPER SOLUTION) USING A SCANNING DENSITOMETER

by

Michael B. Gilbert

An Abstract

TLC was used for qualitative and quantitative analysis of photographic developing agents hydroquinone, metol, phenidone, para phenylenediamine, para aminophenol, ascorbic acid and the oxidized species. Experimental conditions and procedures for reporting R_f values of the aqueous solutions of the developing agent were standardized.

Quantitative TLC was done for hydroquinone. The density maxima obtained from the scanning densitometric trace of the eluted and visualized chromatogram was correlated to the concentration of the hydroquinone. Aqueous solutions

of hydroquinone were prepared at four levels of concentration, three levels of pH and two levels of sodium sulfite concentration for quantitative analysis using TLC.

INTRODUCTION

Recently, in the past twenty years, thin layer chromatography has played an important role in qualitative and quantitative analysis of many types of substances. Substances to be analyzed were complex organic compounds occurring in nature or synthesized by man. TLC's role in biological research has been immense and is an indespensible tool in laboratories in major hospitals.

The chief advantage of TLC, is that it requires very little sample, usually micrograms, to perform the qualitative analysis. In the past ten years, attention in research has been drawn to quantitative analysis of compounds using TLC, but much more success has been gained in the field of gas chromatography. Gas chromatographic instrumentation is complex and much more costly than TLC instruments.

State of the Art

In the 1950's, published papers appeared describing qualitative determinations of photographic developing agents using paper chromatography.[1] Mariani's and Martinelli's article gave the best description of experimental

---

[1]G. Russel, Chemical Analysis in Photography, chapter 8.

conditions they used for paper chromatography.[2] The LuValle and Pannel paper did not describe experimental conditions.[3] Conditions of LuValle's and Pannel's experimentation were not explicitly defined and made it difficult to repeat their results. The author of this paper found two references to published papers involved with qualitative determinations of photo chemicals using paper chromatography.

After World War II, Egon Stahl did intense research in establishing a new type of chromatography. It was similar to the older paper chromatography but had distinct advantages. Thin layer chromatography was coming of age but did not achieve recognition until Stahl's textbook was published in 1961.[4]

> "This book systematizes its voluminous literature and applicable methods so that the text can be used as a laboratory handbook. TLC can be used for organic and inorganic mixtures ranging from 10^{-6} to 10^{-9} gram and has been applied to a large group of complex compounds: drugs, amino acids, natural and synthetic products, vitamins, steroids, dyes, sugars."[5]

---

[2]B. Mariani and P. Martinelli, Sci. Ind. Photog. (Fr.), p. 276.

[3]J.A. Pannel and J.E. LuValle, Analytical Chemistry, Vol. 25, p. 1566.

[4]Thin Layer Chromatography--A Laboratory Handbook, Edited by Egon Stahl,

[5]"Thin Layer Chromatography," Research/Development, pp. 36, 4.

Reference was made in Stahl's textbook to qualitative analysis of photochemicals.[6] In a published paper by J. Eggers, separation and detection conditions were not reported for mixtures of o, m, and para aminophenols and N-methyl-p-aminophenol.[7] Stahl suggests that TLC of phenols and their sulfonic acids should be selected with acidic solvents, of high polarity. Solvent systems suggested by Dr. Baden of the Eastman Kodak research laboratories in Rochester, New York, will be listed in Appendix B.

In qualitative determinations of photographic developing agents using TLC, the R_f value of the developing agent is dependent upon the quality of the solvent system, chromatogram, and activity. Other developing agents should not have an identical R_f value. There are complications; Foremost, the sample of developing agent prepared in aqueous solution requires a preservative or antioxidant to enable it to remain unoxidized and potent. For this reason, sodium sulfite is used in developer formulations. Photographic developer solutions that develop silver halide emulsion require adjustment in a pH range where the developing agent can ionize. It is good sense

---

[6]Stahl, op. cit.

[7]J. Eggers, Photo. u. Wiss., 10, 40 (1961).

to prepare solutions of developing agent and specify conditions of preparation. Once prepared, a small sample size of the photographic developer can be taken by a micro pipet, syringe or streaker, and deposited on the chromatogram. Preliminarily, the sample size taken should contain 1 to 5 ug of the developing agent for qualitative analysis.

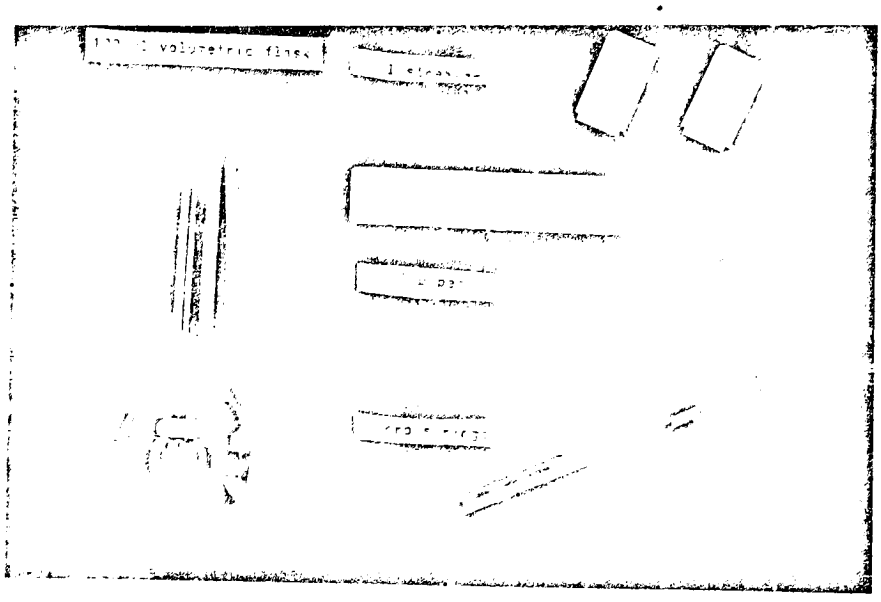


Figure 1

Devices used for depositing samples from prepared solutions

A chromatogram is a coating of silica gel, alumina, diatomaceous earth, kieselgur or oxides of metals that are absorbents coated on a support. This support usually is

glass.

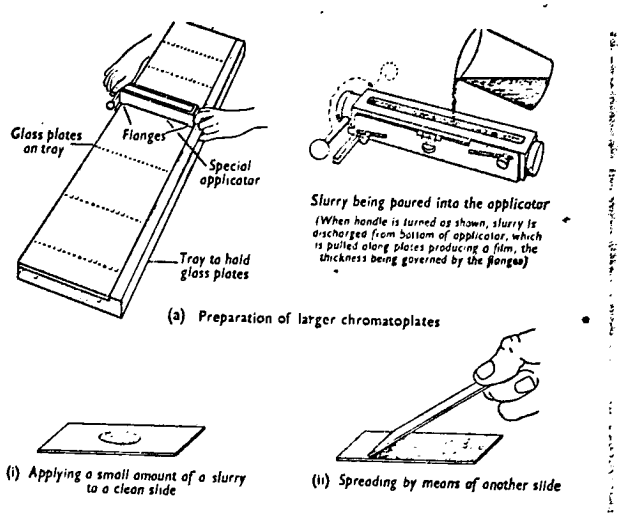


Figure 2[8]

Preparation of Chromatoplates

In the past, coating was done in the laboratory to a specified quality of absorbent and coating thickness. This is a tedious procedure but recently the advent of precoated substrates of silica, alumina and polyamide layers have eliminated this burden.

---

[8]David Abbott, An Introduction to Chromatography, p. 26.

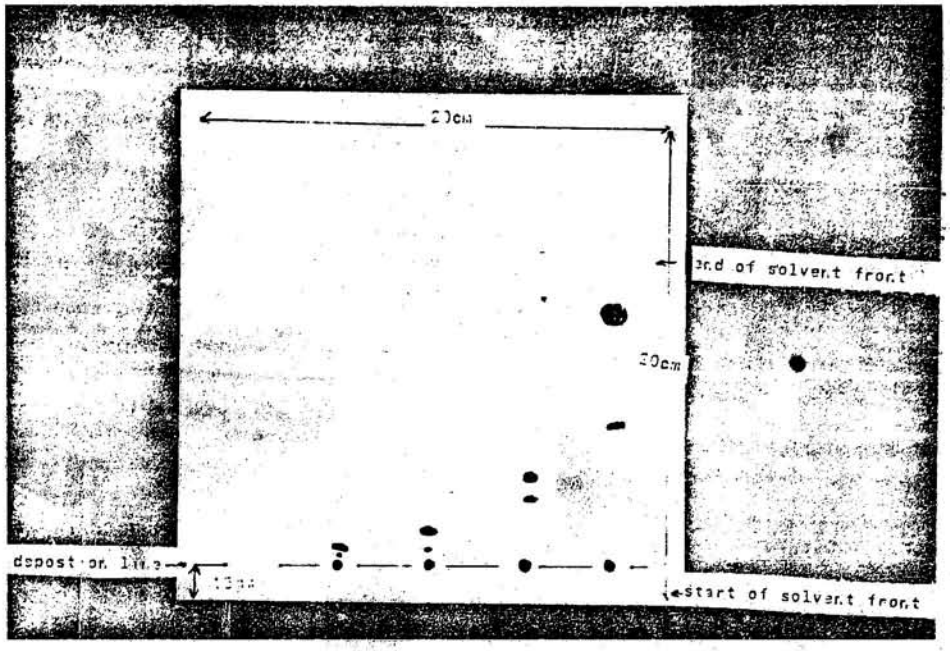


Figure 3

Dimensions of Precoated Chromatogram

Prior to sample deposition, it may be necessary to activate the chromatogram by heating it in an oven. Water from the atmosphere can be absorbed by the chromatogram substrate and form an immobile phase. It will also affect adsorption properties of the substrate to the deposited sample. Once the sample is deposited on the chromatogram substrate, it is dried. Forced (heated) air drying may oxidize the developing agent.

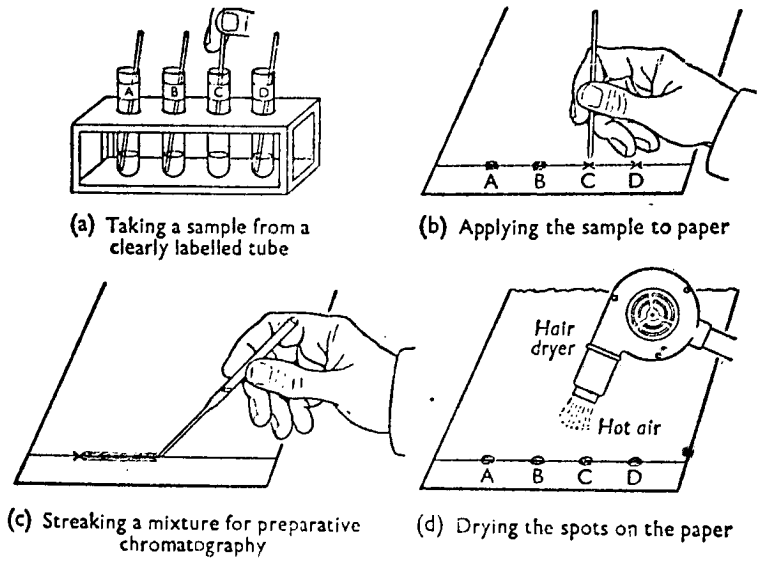
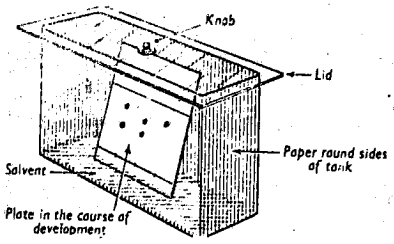


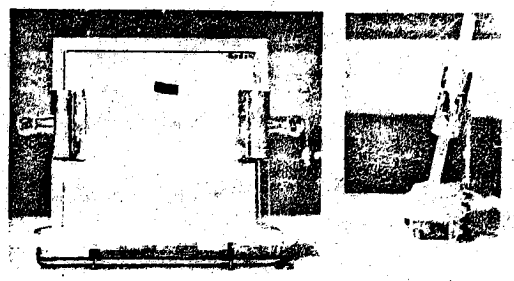
Figure 4[9]

Application of Samples to Thin Layer Chromatogram

Elution in the appropriate solvent system is the next step. The chromatogram is placed in the confine of a vessel that has the solvent. This will ensure an atmosphere saturated with solvent vapors. Otherwise there will not be an even front travel.

[9]Ibid., p. 10.





a) Tank[10] b) Chamber Plates

Figure 5

For precoated flexible supports, chamber plates have better properties over other closed vessels. During the elution, the apparatus should be isolated from any temperature or humidity changes since this will affect the rate and evenness of travel of the solvent system and the adsorption of the developing agent to the chromatogram substrate. The elution of the solvent system in the chamber plate is ascending. After a period of time, when the solvent front has migrated up the chromatogram a distance, the elution is stopped by removing the chromatogram from the confines of the apparatus. The position of the solvent front is noted.

Visualization of a compound is necessary if it is not visible. Photographic developing agents can be made visible by spraying the chromatogram with ammoniacal .1N

---

[10]Ibid., p. 28.

silver nitrate. Spraying with an atomizer will make developing agents visible so that measurements for R_f values can be made. Application of the visualization reagent for qualitative purpose should be sufficient to make the substances visible. If too much visualization reagent is applied, the sharpness will decrease. For quantitative purposes, little is found in the literature about the amount of visualizing reagent needed.

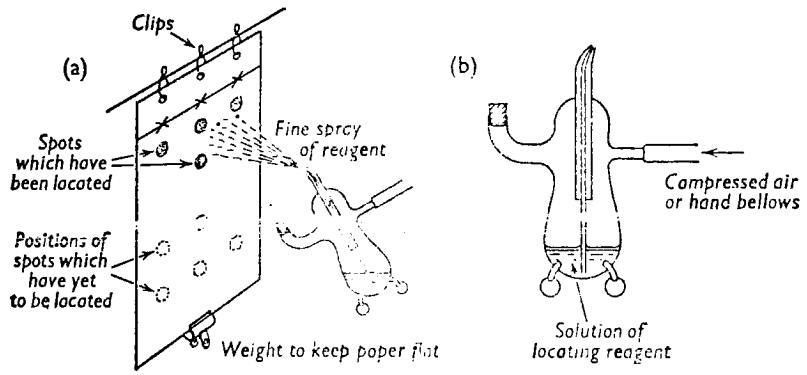


Figure 6[11]

Application of Visualization Reagent with Atomizer

Before spraying with the visualization reagent and taking measurements, the chromatogram should be air dried. Care was taken when mixing and spraying the silver nitrate. Spraying was done under a blower hood. When finished, the excess silver nitrate was disposed of immediately.

[11] Ibid., p. 16.

R_f values for each developing agent can be determined by measuring the ratio of distance the developing agent traveled after elution and the distance the solvent front traveled.

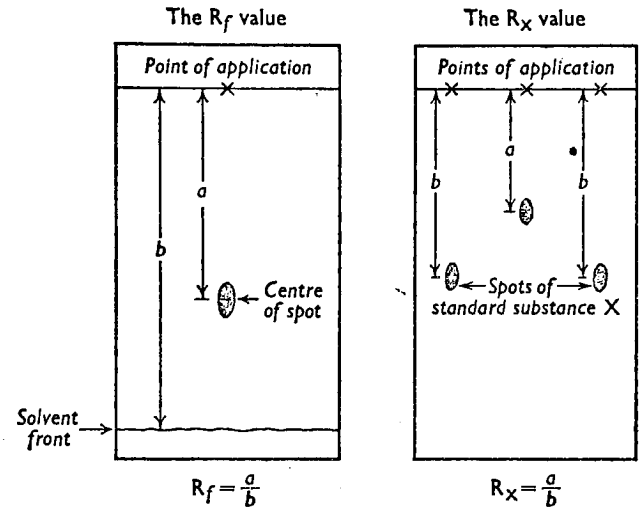


Figure 7[12]

R_f and R_x Values

A variety of visualization reagents are available.[13]

Two dimensional TLC can be used to improve separation of developing agents prepared in one solution. Some developing agents may have similar R_f values for a particular chromatogram substrate and solvent system. Two dimensional TLC is done by allowing the chromatogram (with a sample

---

[12] Ibid., p. 17.

[13] E.K. Bibliography on visualization reagents.

spotted in the lower left hand corner) to elute in the ascending direction for a period of time. Afterwards, the chromatogram is removed, dried and rotated 90° counter-clockwise and placed in another solvent system. Elution in the second solvent system is continued for a period of time and then stopped. Two R_f values, for the first and second elutions, are used to specify the x and y coordinates on the chromatogram where the developing agents separated to.

Steps in Two Dimensional Chromatography:

1. sample preparation and deposition
2. activation
3. first elution
4. dry
5. rotate 90° counter-clockwise
6. second elution
7. dry
8. visualization

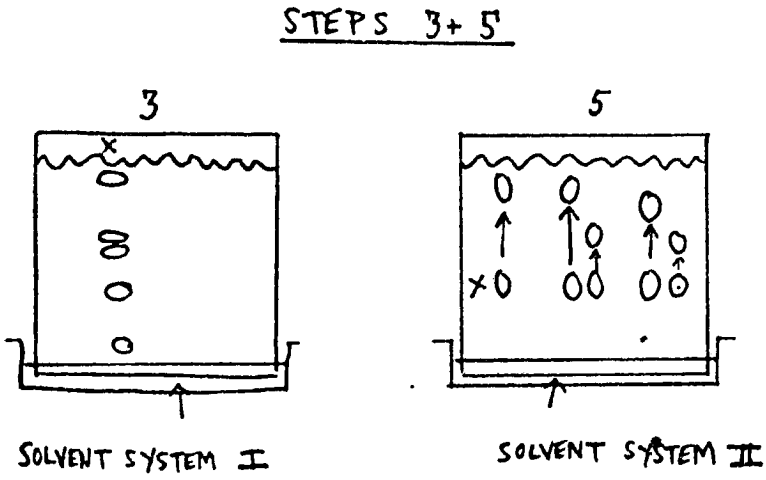


Figure 8

Two Dimensional Chromatography

"While TLC qualitative analysis are relatively simple to do using standards, visualization reagents and R_f values, obtaining quantitative information is considerably more difficult...By completely standardizing conditions of sample size and TLC elution, conditions and calibration standards can be used to provide calibration curves of spot area versus amount of component. The amount of the material in a spot can also be determined with a density measurement obtained by photometric or photographic means. The most accurate results, however, are those obtained by extraction of the material in the spot from the adsorbent using as solvent and then determining the amount colorimetrically or spectrophotometrically."[14]

TLC densitometry has been advanced by introduction of

[14]F.W. Karnsk, "Thin Layer Chromatography," Research/Development, 4.

the split reference beam. This has overcome many of the variables leading to inaccuracies. Determinations can be made with relative standard deviations of two to three per cent. Quantitative evaluation of thin layer chromatograms by conventional methods without extraction of separated substances are discussed in Stahl's textbook, Thin Layer Chromatography--A Laboratory Handbook.

Theory of Thin Layer Chromatography

In TLC, separation of compounds is obtained as it travels with a migrating solvent through a thin layer of adsorbent material coated on a glass or flexible support. Separation of compounds is due to adsorption, partition or reverse phase mechanisms.

The most common mechanism is adsorption which is useful for separation of lipophilic organic compounds of medium or low polarity. Silica gel and alumina oxide are used for adsorption chromatography. The material being separated is proportioned between the migrating solvent system and the active adsorption sites in the adsorbent. Highly polar compounds will be strongly adsorbed. The polarity of organic compounds, such as photographic developing agents, will be established by the type and number of functional groups that have the ability of forming bonds with hydrogen.

Partition TLC is useful for polar, water soluble,

inorganic compounds. Unactivated silica gel, carrying an adsobed phase of water, can be used. In partition TLC, a proportion is established between the migrating solvent system and the adsorbed stationary mobile phase. Separation of substances is similar to factors governing a separatory funnel extraction, where relative solubilities (hence, the partition coefficient) of the substances determine this proportion.

Reverse phase TLC is efficient in separating homologous lipophilic compounds. This is a less frequently used mechanism.

Theory

Consideration of these mechanisms is necessary in selecting the right migrating solvent (solvent system), chromatogram substrate, pretreatment (activation) and elution conditions.[15]

The R_f value of a substance is a number derived for a compound.

$$R_f = \frac{\text{distance of center of spot from origin}}{\text{distance of solvent front from origin}}$$

R_f values in adsorption TLC depend on factors which must be controlled.

1. Quality of adsorbent- Silica gel, alumina and kieselgur are of many substrates to choose from.[16]
2. Thickness of adsorbent layer- controlled by the coating operation.
3. Degree of activity of adsorbent- pretreatment of chromatogram by placing in dry heated oven or soaking the chromatogram in a treatment solution to adjust pH or chemically impregnating the chromatogram can be done.
4. Quality and nature of solvents used- a variety of solvent systems with one or more solvent components

---

[15]"Thin Layer Chromatography with Eastman Chromagram Sheet and Developing Apparatus," Kodak Publications, reprint E, 1972.

[16]Reiner Lapp, "Brinkman Bibliography for TLC," 1965.

with many combinations exist.[17]

5. Degree of chamber saturation- There are different vessels of varying size characteristics. Ideally the atmosphere surrounding the chromatogram should be saturated with solvent vapor.
6. Temperature- This will affect the adsorption of a compound as well as rate of travel of the solvent system.
7. Running distance- The distance the solvent system travels should be kept constant by recording the time of elution and travel distance.
8. Technique of elution- There is a choice of development apparatus for ascending, descending or horizontal development.
9. Amount of sample- 1 to 5 ug samples are recommended as a starting point. Tailing is observed with samples of greater concentration.
10. Impurities present in sample mixture- Water is an impurity that will affect the degree of activity of adsorption sites.
11. Visualization- This is done to render an invisible compound visible. Attention is drawn to the problem of tailing. At higher concentrations, tailing

---

[17]Eastman Organic Chemical Catalogue #46, pp. 391-392, 1971, published by the Eastman Kodak Company, Rochester, New York.

is a visual effect which was observed to be an unsharp separation of the compound after elution. Some compounds are not visible after elution and need to be made visible. There are a variety of visualization reagents available for invisible compounds. They react and form a colored product.[18] As mentioned in the "State of the Art," application of the visualization reagent will have significant contribution to the sharpness of the detected compound after elution.

In TLC a substance is separated on the chromatogram as it is being transported by a mobile phase of a solvent system. TLC can bring about a separation by an adsorption or partition mechanism. The tailing effect for adsorption TLC can be explained in terms of a Langmuir and Freundlich adsorption isotherm. These adsorption isotherms dictate the condition of the amount of a substance adsorbed by an adsorbent.

---

[18]"Eastman TLC Visualization Reagents and Chromatographic Solvents," Kodak Publications JJ-5, July, 1971, p. 8.

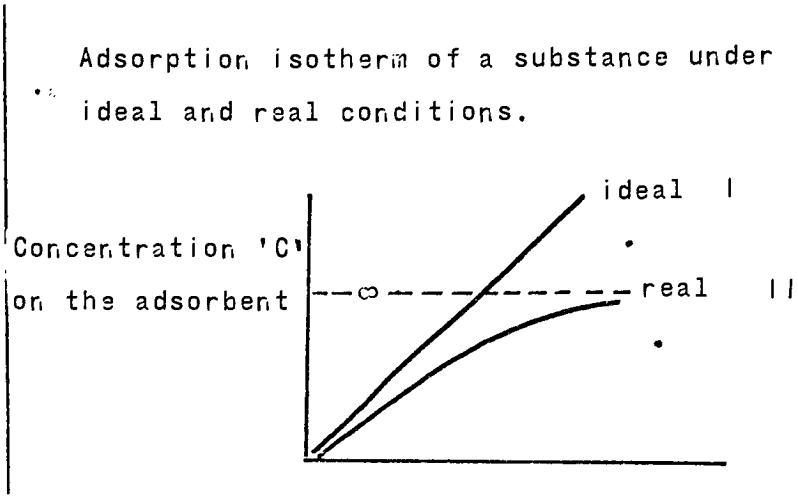


Figure 9

Adsorption isotherm of a substance at different levels of concentration

Freundlich and Langmuir isotherms:

1) $M = aP^b$ (Freundlich)

2) $M = dKP/(1+KP)$ (Langmuir)

M-is mass of adsorbate/ mass of adsorbent

P-equilibrium pressure of adsorbate

a, b, and k are constants characteristic of particular adsorbents which are particularly temperature dependent.[19]

---

[19]Bladel and Meloche, Elementary Quantitative Analysis, second edition, p. 599.

If a substance were deposited on a chromatogram, eluted, and visualized and the image of the visible substance was scanned with a densitometer, a trace would be obtained. Traces 5, 6, 7, and 8 (in figure 9) are traces of a chromatogram with samples of hydroquinone at .5, 1.0, 5.0 and 10.0 g/l concentration after elution and visualization with .1N ammoniacal siver nitrate. (see figure 10).

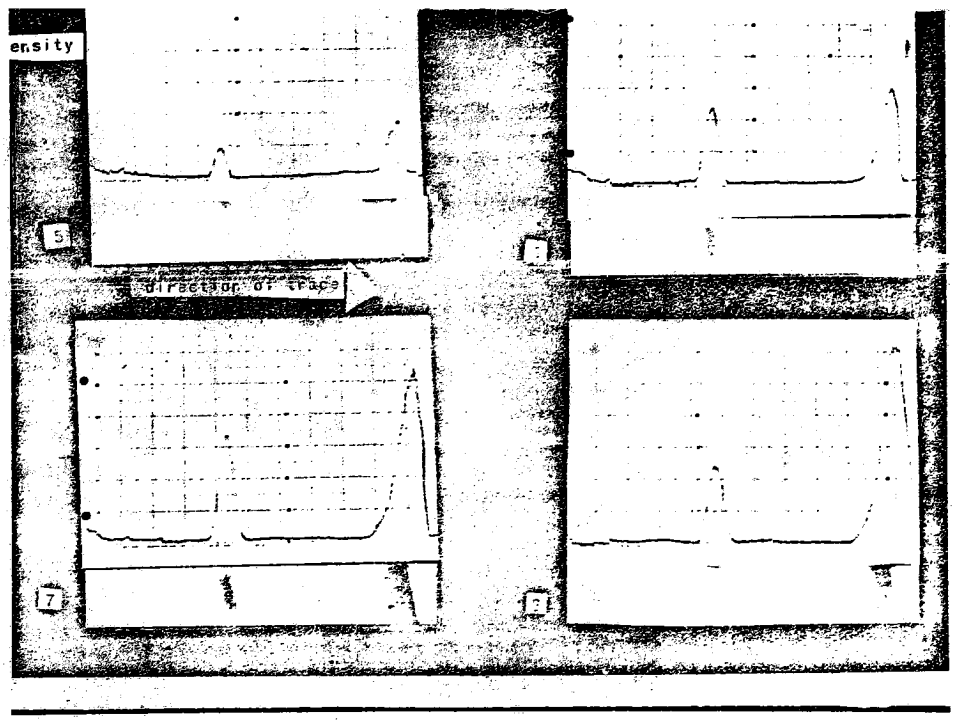


Figure 10

Densitometric Traces

The non-symmetrical distribution of the separated substance at higher concentration on the chromatogram was

seen on the densitometer trace as a skewed gaussian distribution. Also, it was noticed that the maximum density of the trace was increasing with greater concentration of hydroquinone.

The reason for this, as explained in terms of the adsorption isotherm, is that at higher concentrations there are not enough active adsorption centers to separate out the compound. Under ideal conditions where there are enough adsorption centers, the compound will be separated out sharply. However, under real conditions, there is a decreasing linearity. In other words, there is a maximum concentration of substance which the adsorption centers can adsorb. Beyond this concentration, tailing will exist.

In the partition mechanism of separation, much the same is true for the tailing effect. A partition mechanism involves two phases; one mobile and the other immobile. If the substance is not saturated in the mobile phase, there will be a sharp separation. The saturated state of a solute in a solvent is dictated by the partition coefficient. When there is a saturated state for the substance in the mobile phase, the substance will separate from the rest of the migrating substance until equilibrium of the partition coefficient in the mobile phase is restored.

Inorganic salts, such as sodium sulfite, act as

hydrophillic or water attracting particles. This will affect the equilibrium of water in the different phases. R_f values obtained under these conditions will be with error due to difficulty of measurement with tailing effects.

Two different substances will separate out to different positions on the chromatogram during elution if there is difference in the adsorption properties or partition coefficient with a particular solvent system and chromatogram substrate.

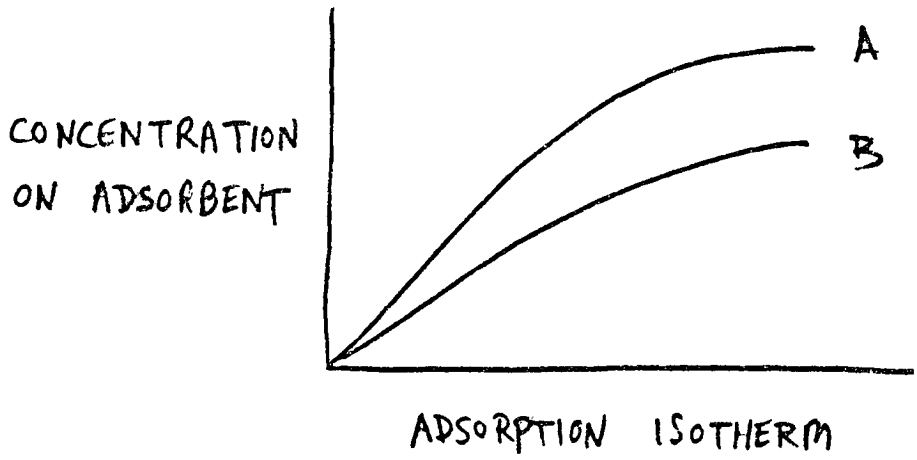


Figure 11

Adsorption isotherm of two different substances

A system of separation of class of compounds can be

devised by choosing the appropriate mechanisms which will bring about the separation.

In TLC, a variety of mechanisms for separation can be controlled by the choice solvent system, quality of adsorbent and degree of activation. Activation is the process of heating the chromatogram in a dry oven for a period of time to dry off the water from the atmosphere which can be absorbed by the silica gel. Water will be adsorbed at the activation sites. This means the degree of the activity will be determined by the amount of water present in the atmosphere or in the solvent system. For photographic developing agents and other non-polar organic compounds a variety of non-polar solvents are used for a solvent system. If the mechanism of separation in TLC is primarily adsorption, the chromatogram will be activated and the solvent system will contain little or no water. There are other substrates besides silica but when activated, do not exhibit as much adsorptive properties. By not activating silica and other chromatograms and poisoning the solvent system with more polar solvents such as water, the separation can be shifted to a partition mechanism.

> "Generally, hydrocarbons are adsorbed slightly, if at all, and thus, migrate fastest. Unsaturated hydrocarbons are more strongly adsorbed the more double bonds they contain. Hydrocarbons containing conjugated double bonds are strongly adsorbed than those having a system of isolated double bonds. If functional groups are introduced into a hydrocarbon, the adsorption

affinity is increased in the following sequence: $-CH_3$, O-Alkyl, $>C=O$, $-NH_2$, OH, COOH."[20]

[20]Stahl, op. cit., p. 135.

OBJECTIVE OF THESIS

TLC was used as a qualitative and quantitative analysis of photographic developing agents prepared in aqueous solution with specified levels of sodium sulfite concentration and pH. There were three specific objectives.

I. R_f values of developing agents- R_f values were tabulated for aqueous solution of developing agents metol, hydroquinone, hydroquinone monosulfonate, phenidone, ascorbic acid, CD-3, para aminophenol, para phenylenediamine HCL and amidol. Two different solvent systems were used.

II. Two dimensional TLC- Hydroquinone, phenidone, metol and CD-3 developing agents were mixed into one solution and two dimensional TLC was used to separate the developing agents from one another.

III. Quantitative TLC- TLC was used for quantitative analysis of hydroquinone prepared at four levels of concentration, three levels of pH and two levels of sodium sulfite concentration. The density maxima $D_m(\#)$ obtained after visualization and densitometric scanning was correlated to the concentration of hydroquinone prepared in developer solutions.

OBJECTIVE I

Procedure

1. Two per cent solutions of metol, hydroquinone monosulfonate, hydroquinone, phenidone, ascorbic acid, CD-3, para aminophenol HCL, para phenylenediamine HCL and amidol were prepared by adding .2 g of each developing agent to a 100 ml volumetric flask which contained 5g of sodium sulfite and approximately 80 ml of water. The CD-3 solution contained .2g of sodium sulfite. A phenidone/ ascorbic acid solution was prepared.
2. The pH of the solution was adjusted to 10.0 with 6N sodium hydroxide. The amidol solution was adjusted to a pH of 7.0.
3. The volumetric flasks were brought up to volume by adding a solution of water and sodium sulfite at 1g/l. Afterwards the solutions were stoppered.
4. 5 ul samples of each solution was drawn up by a streaker and deposited on an unactivated silica gel chromatogram. The chromatogram was precoated and manufactured by Eastman Kodak. The samples were placed 15 mm from the bottom edge and 2.5 cm apart from one another.

This accomodated eight samples deposited on the 20 cm wide thin layer chromatogram.

5. The sample was placed in the chamber plate and inserted into a fish tank that contained a reservoir of the solvent system. The solvent system contained n-butanol; acetic acid and water in relationship of 80:5:15 parts by volume (v/v). 150 ml of the solvent system was present in the reservoir.
6. After elution of the chromatogram commenced, it was stopped five and a half hours later. Temperature during the elution and replications was between 70° and 78° F. with a relative humidity between 30 and 50 %.
7. The chromatogram was withdrawn from the chamber plate apparatus and dried in an oven at room temperature.
8. Visualization of the invisible developing agents that were separated during the elution was done by spraying with a .1N ammoniacal silver nitrate solution. This visualizing reagent was prepared by adding .75ml of concentrated ammonium hydroxide to 30ml of .1N silver nitrate. The chromatogram was sprayed under a blower hood in a repeating pattern with an atomizer hooked up to a five pound pressure line. Afterwards, the visualizing reagent was diluted and dumped because of the danger of instability of ammoniacal silver nitrate.
9. Measurements for determination of R_f value were taken

with a metric rule after the chromatogram had dried and before turning an over-all brown. This browning occured about twenty-four hours after visualization.

10. Steps four through nine were repeated with solutions one through eight to obtain an estimate of experimental error.

11. Solutions one through eight were saved and 50ml of the photographic developing agent solutions were placed in an open 100ml beaker for over twenty-four hours. This was done to encourage aerial oxidation of the photographic developing agent.

12. TLC of the photographic developing agents one through eight and their oxidized species was done by repeating steps four through nine.

13. A benzene:methanol solvent system (90:10) v/v was prepared to replace the n-butanol:acetic acid:water solvent system (80:5:15). Prior to deposition of fresh samples of photographic developing agents one through eight, the silica gel chromatogram was activated. Activation was achieved by placing the silica gel chromatogram in a dry oven at 100° C. for fifteen minutes.

Observations

1. The phenidone, amidol and sodium hydroquinone monosulfonate solutions changed color after being

deliberately oxidized. Also an additional specie was formed and an additional R_f value was calculated for each specie formed.

2. The benzene:methanol solvent system took one hour to elute 10 cm up the chromatogram. The R_f values were generally lower than the n-butanol:acetic acid:water solvent system and were much sharper visually.

3. The pH of n-butanol:acetic acid:water solvent system was 2.32 and the pH of the benzene method solvent system was 6.6

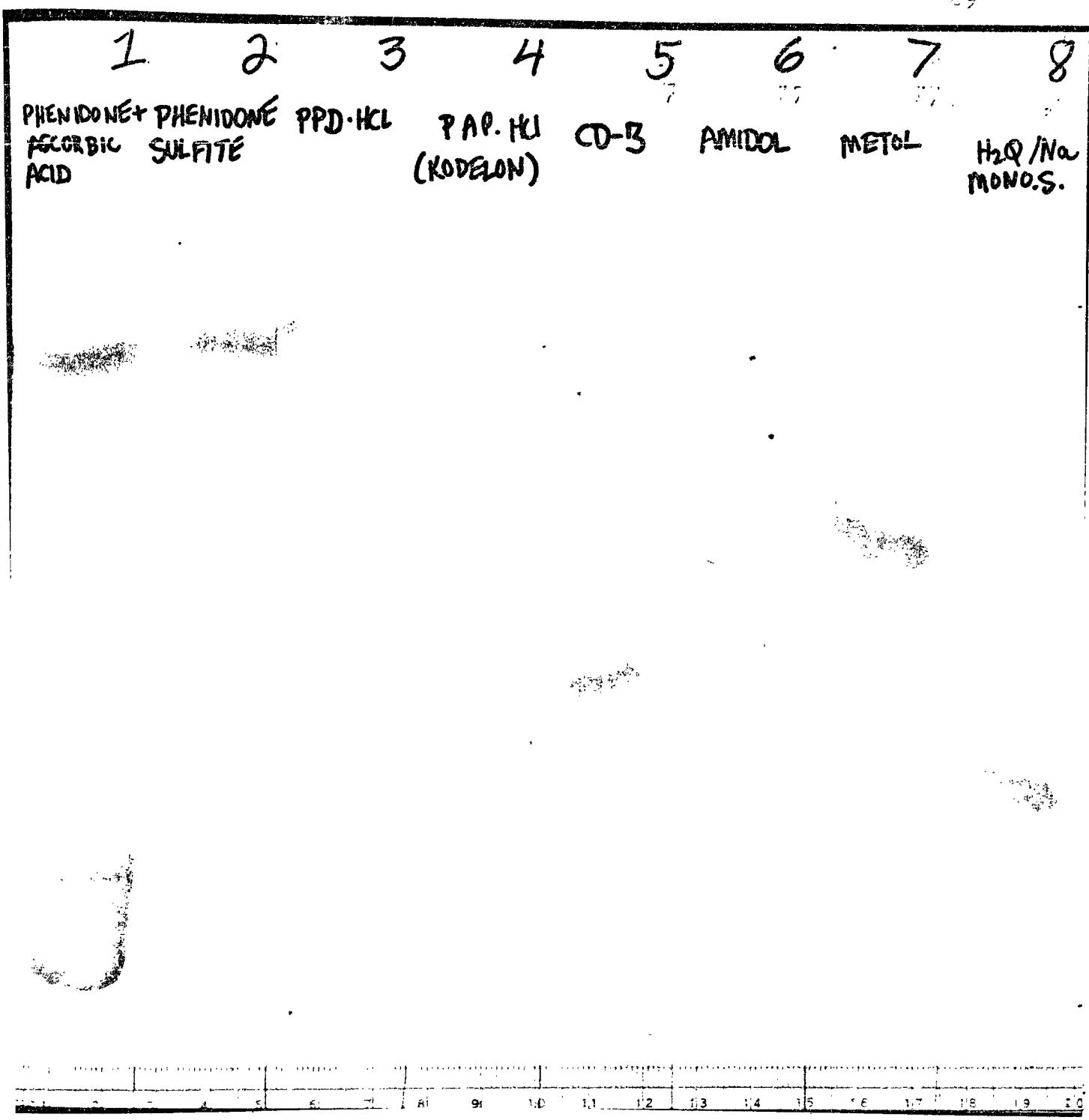


Figure 12

TLC of photographic developing agents 1-8 prepared in aqueous solution for with inactive silica gel used with the n-butanol:acetic acid: water solvent system

Data and Calculations

1. R_f values of photographic developing agents 1-8 (see figure 12).

   substrate- silica gel, inactive

   sample deposition- 5 ul streaker

   solvent system- n-butanol:acetic acid:water (80:5:15)

   elution time- five and a half hours .

solution#	developing agent	R_f value	replicate	average
1	phenidone (2g/l)	.83	.83	.83
2	phenidone (2g/l) and ascorbic acid (10g/l)	.83 .04	.84	.84
3	para phenylene-diamine HCL (2 g/l)	.42	.44	.43
4	para aminophenol HCL (2g/l)	.62	.61	.62
5	CD-3 (2g/l)	.40	.40	.40
6	amidol (2,4-diaminophenol)	.30	.29	.30
7	metol	.58	.57	.58
8	sodium hydroquinone monosulfonate	.32	.31	.32

2. R_f values of photographic developing agents deliberately oxidized by exposure to atmosphere.

solution #	developing agent	R_f values	oxidized species
1	phenidone (2g/l)	.87	.27
2	phenidone (2g/l) and ascorbic acid	.84 .08	
3	para phenylene-diamine HCL	.43	
4	para aminophenol HCL	.71	
5	CD-3	.43	.21
6	amidol		.04
7	metol	.63	.34
8	sodium hydroquinone monosulfonate	.34	.01

3. R_f values of photographic developing agents 1-8

substrate- silica, activated

sample deposition- 5 ul streaker

solvent system- benzene:methanol (90:10) v/v

elution time- one hour

solution #	developing agent	R_f value	replicate	average
1	phenidone (2g/l)	.45	.46	.46
2	phenidone (2g/l) and ascorbic acid	.44 .00	.46 .01	.45 .01
3	para phenylene-diamine HCL	.34	.35	.35
4	para aminophenol HCL	.32	.33	.33
5	amidol	.07	.07	.07
6	CD-3	.41	.42	.42
7	metol	.37	.38	.38

Conclusion

1. The n-butanol:acetic acid:water solvent system (80:5:15), took five and a half hours to migrate up the chromatogram. This was four and a half hours longer than the benzene:methanol (90:10) solvent system. The advantage of the butanol solvent system was that R_f values were obtained for oxidized species of hydroquinone, metol, amidol, CD-3 and phenidone when prepared in aqueous solution form in the presence of sulfite.
2. The oxidation of a photographic developing agent in aqueous solution with sodium sulfite probably resulted in the formation of a sulfonic acid of the developing agent. Hydroquinone, when oxidized by oxygen, will form a para benzo quinone. However, sodium sulfite will react with it to form a colorless weak developing agent.

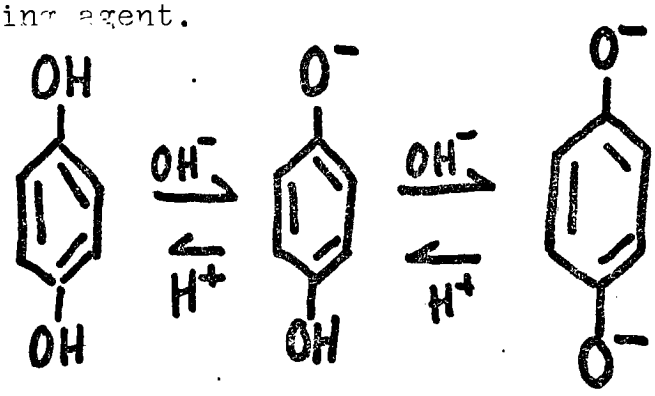


Figure 13

Step wise ionization of hydroquinone to a quinone.

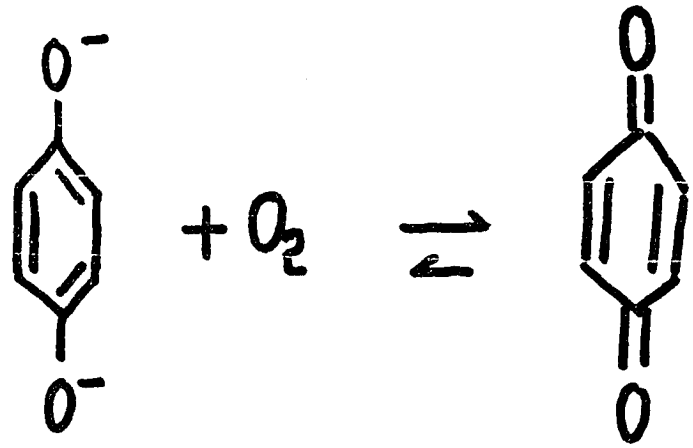


Figure 14

Oxidation of hydroquinone to a para benzo quinone

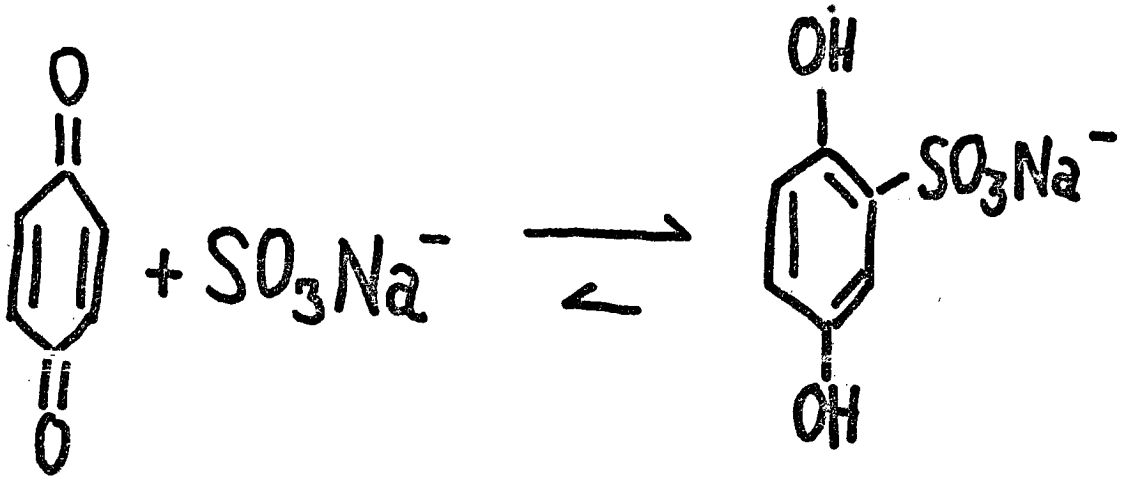


Figure 15

Formation of hydroquinone monosulfonate

3. A similar mechanism is possible for phenidone, amidol and CD-3. The phenidone/ascorbic acid developer did not form any additional species as compared to the phenidone/sodium sulfite solution. This indicates that ascorbic acid is a more effective anti-oxidant than sodium sulfite.

4. The sodium hydroquinone monosulfonate formed another species when oxidized. The author has not been able to attribute the quality of the species of the hydroquinone monosulfonate. It is possible that it is hydroquinone disulfonate.

5. The benzene:methanol solvent system gave a different set of R_f values for developing agents 1-8. Independent testing of oxidized species of those developing agents did not reveal the oxidized species by separating them from the unoxidized species of developing agents. This was probably due to the fact that the benzene:methanol solvent system was at a higher pH than the other butanol:acetic acid:water solvent system.

6. This confirms Stahl's statement that more acidic polar solvent systems could be more successful in separating sulfonic acids of developing agents.[21] It is for this reason that the n-butanol solvent system was superior to the less polar and less acidic benzene:methanol solution.

7. The Eastman Kodak Company prepared a publication on "Separation of Photographic Developing Agents Using the Eastman Chromagram System." They reported approximate R_f values for the following developing agents using an activated silica gel chromatogram and a

---

[21] Ibid., p. 369.

benzene/methanol (90:10 v/v) solvent system.

	developing agent	R_f value
a.	2,4-diaminophenol sulfate	.00
b.	hydroquinone	.15
c.	N-methyl-p-aminophenol sulfate (metol)	.25
d.	1-phenyl-3pyrazolidone (phenidone)	.30
e.	4,4'-dimethyl-1-phenyl-3-pyrazolidone	.40

The elution time was thirty minutes which probably accounted for the difference in R_f values. Also, the silica sheet used in Objective 1 was not activated.

OBJECTIVE II

Procedure

1. Two per cent solutions of metol, hydroquinone, phenidone and CD-3 were prepared in a manner similar to the preceeding procedure in part 1, section 1.
2. 25 ml of each developer solution was placed into a 100 ml volumetric flask and mixed together.
3. 5 ul of the solution was drawn by a micro-pipet and the spot was placed on an activated silica chromatogram 20 mm from the bottom edge and 60 mm from the left edge. The 5 ul sample was deposited on the chromatogram twenty minutes after the chromatogram had been activated.
4. A benzene/methanol (90:10) v/v solvent system was prepared and 150 ml of the solvent system was placed in the reservoir.
5. The spotted chromatogram was placed in the chamber plate and eluted for one hour in the first solvent system.
6. After elution, the distance the even solvent front traveled, was measured.
7. The chromatogram was air dried for fifteen minutes.

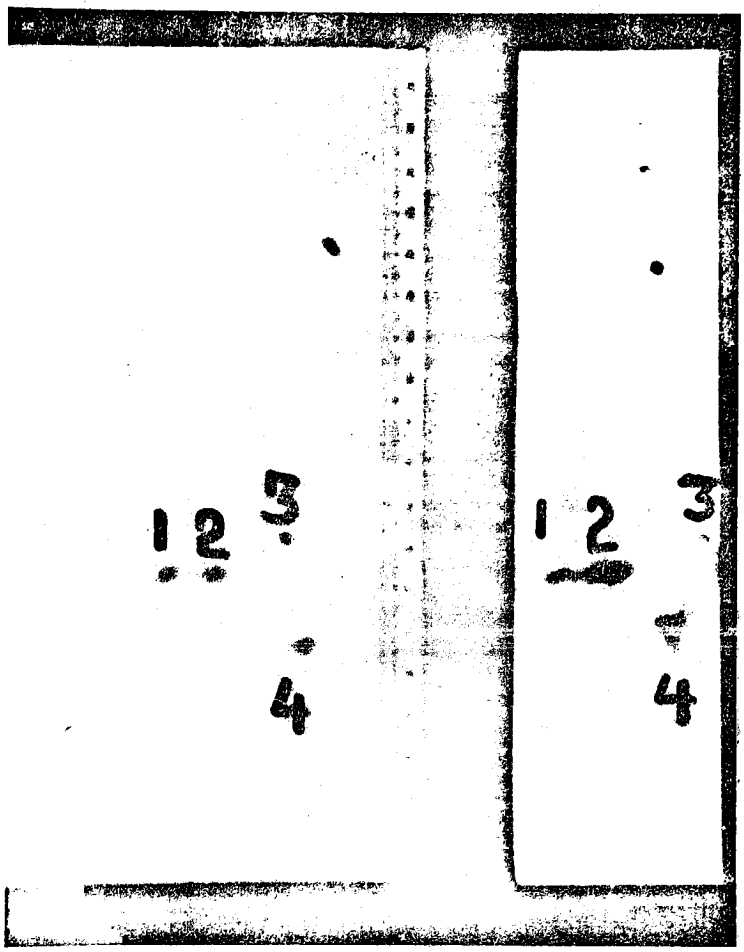
8. Following the 90° counter-clockwise rotation of the chromatogram, the chromatogram was placed in the second solvent system (n-butanol:acetic acid:water) and eluted for one and a half hours. The distance solvent system two traveled was measured.
9. The chromatogram was dried for twenty minutes and visualized with the ammoniacal silver nitrate visualization reagent.
10. After the chromatogram was dry, two dimensional R_f values were measured.

Observations

1. The separations of the developing agent are sharp and separate from one another. There was little problem in recognizing them since each was separated from one another.
2. There was a slight uneven solvent front travel during the second elution in both trials.
3. Before performing the two dimensional TLC, it was helpful in spotting an additional sample of substances on a margin of the chromatogram. This was not to be run with the other sample during the second elution. By removing the margin sample from the rest of the chromatogram after first elution and visualizing it before the second elution of the other half of the chromatogram, it was possible to monitor the progress

of the separation of substances during the first elution.

4. Figure 16 shows the chromatogram in two parts. Part a is the chromatogram that had the solution of four developing agents spotted in the lower left hand corner, eluted in the verticle direction with the benzene/methanol solvent system, rotated 90° counterclockwise and eluted in the second solvent system with the n-butanol:acetic acid:water solvent system, and then visualized. Part b was the chromatogram that had four separate samples of each developing agent deposited 20 mm from the bottom edge and eluted vertically with the benzene/methanol solvent system only. Then it was visualized.



a) b)

Figure 16

Two Dimensional TLC

Data and Calculations

R_f coordinates for developing agents:

	(1) $R_{f(x)}$	replicate $R_{f(x)}$	average. $R_{f(\overline{x})}$	(1) $R_{f(y)}$	replicate $R_{f(y)}$	average $R_{f(\overline{y})}$
1.	.28	.29	.29	.74	.76	.75
2.	.38	.39	.39	.50	.52	.51
3.	.43	.44	.44	.69	.73	.71
4.	.36	.40	.38	.34	.35	.35

1. hydroquinone, 2. metol, 3. phenidone, 4. CD-3.

Conclusion

1. Two dimensional TLC can improve the separation of photographic developing agents prepared in a solution and deposited on a chromatogram.
2. Elution time with the two solvent systems required only three hours.
3. Reversing or changing the order of elution will also change the R_f coordinates. Prior to the first elution, the chromatogram was activated. After the first elution, the chromatogram was not activated but air dried in preparation for the second elution. It was possible that there was a stationary methanol phase during the second elution. It appeared that benzene or methanol left over from the first elution did not effect the separation of the developing agents during the second elution to any great degree.

In other words, if the second elution was continued to five and a half hours with the n-butanol:acetic acid:water solvent system, the R_f values would be approximately the same (as Objective I).

4. Providing there is good even solvent front travel during both elutions, experimental error should be small.

OBJECTIVE III

Procedure

1. A silica gel chromatogram, 20X20 cm, was used for depositing eight samples of the hydroquinone developer solution, 2.5 cm apart from one another and 2.0 cm from the bottom edge of the chromatogram.
2. Solutions of hydroquinone for quantitative TLC were prepared from stock solutions of hydroquinone and sodium sulfite. By adding proper volume of each stock solution, the concentration of hydroquinone was set at one of the four levels.
3. The four levels of hydroquinone were set at .5, 1.0, 5.0 and 10.0 g/l. The sodium sulfite concentration was set at 10 g/l and 100 g/l. pH was adjusted to 9, 11, or 13 with 6N sodium hydroxide after the solution was brought up to 90% of the final volume. A 1% aqueous solution of sodium sulfite was used to bring the developer solution up to final volume.
4. The hydroquinone stock solution was prepared in the following manner:
   a 500 ml distilled water at 125° F.
   b 10 g sodium sulfite (dessicated)

c 20 g hydroquinone

d 10 g sodium sulfite

e bring level to 1l volume with .1% aqueous sodium sulfite solution

f stopper and record date of preparation

5. The sodium sulfite stock solution was prepared in the following manner:

a 750 ml distilled water at 75° F.

b 200 g of sodium sulfite

c bring level to 1l volume

6. Table I was constructed to indicate the proper volume of stock solutions of hydroquinone (I) and sodium sulfite (II) to be used to formulate developer solutions used for runs 1-49.

Table I

run	stock solution I	stock solution II	adjust pH to
1,5	2.50 ml	4.75 ml	9
2,6	5.00 ml	4.50 ml	9
3,7	25.00 ml	2.50 ml	9
4,8	50.00 ml	0.00 ml	9
9,13	2.5 ml	49.75 ml	9
10,14	5.0 ml	49.50 ml	9
11,15	25.00 ml	47.50 ml	9
12,16	50.00 ml	45.00 ml	9
17,21	2.5 ml	4.75 ml	11
18,22	5.0 ml	4.50 ml	11
19,23	25.0 ml	2.50 ml	11
20,24	50.00 ml	0.00 ml	11
25,29	2.5 ml	49.75 ml	11
26,30	5.0 ml	49.50 ml	11
27,31	25.00 ml	47.50 ml	11
28,32	50.00 ml	45.00 ml	11

Table I (cont'd)

run	stock solution I	stock solution II	adjust pH to
33,37	2.5 ml	4.75 ml	13
34,38	5.0 ml	4.5 ml	13
35,39	25.0 ml	2.5 ml	13
36,40	50.00ml	0.0 ml	13
41,44	2.5 ml	49.75 ml	13
42,45	5.0 ml	49.5 ml	13
43,46	25.0 ml	47.5 ml	13
44,47	50.0 ml	45.0 ml	13

The volume of stock solutions I and II were added by buret for runs 1-48.

7. Table II was the planned factorial experiment which listed each factor and level of hydroquinone, sodium sulfite and pH. In addition, it also listed what order the experimental runs were to be made.

Factorial Experiment

Table II

		10.0			100.00		
		9	11	13	9	11	13
HYDROQUINONE	.5	1	17	33	9	25	41
		5	21	37	13	29	45
	1.0	2	18	34	10	26	42
		6	22	38	14	30	46
	5.0	3	19	35	11	27	43
		7	23	39	15	31	47
	10.0	4	20	36	12	28	44
		8	24	40	16	32	48
day of run		1	2	3	1	2	3

sodium sulfite

pH

8. For each day, sixteen runs were done. There were two chamber plate apparatuses. The first chamber plate was confined to do runs that had 10 g/l concentration of sodium sulfite. The second chamber plate was confined to do runs that had 100 g/l of sodium sulfite concentration. A chromatogram placed in each chamber plate accomodated eight runs. There were four runs that had their replicates in addition to being on the same chromatogram. This was the state of the art in quantitative TLC.
9. Eight solutions were prepared twenty-four hours in advance to sample deposition onto the chromatogram. From each solution bottle containing 100 ml of the hydroquinone at the specified level with sodium sulfite and pH, a few drops were transferred to the streaker trough.
10. The streaker was the device used to deposit a 5 ul sample on the chromatogram. Deposition was achieved by placing the streaker in the trough which contained the solution. It was lifted out by tilting the streaker out of the solution. A chem wipe towel was used to wipe off the excess solution from the teeth of the streaker.

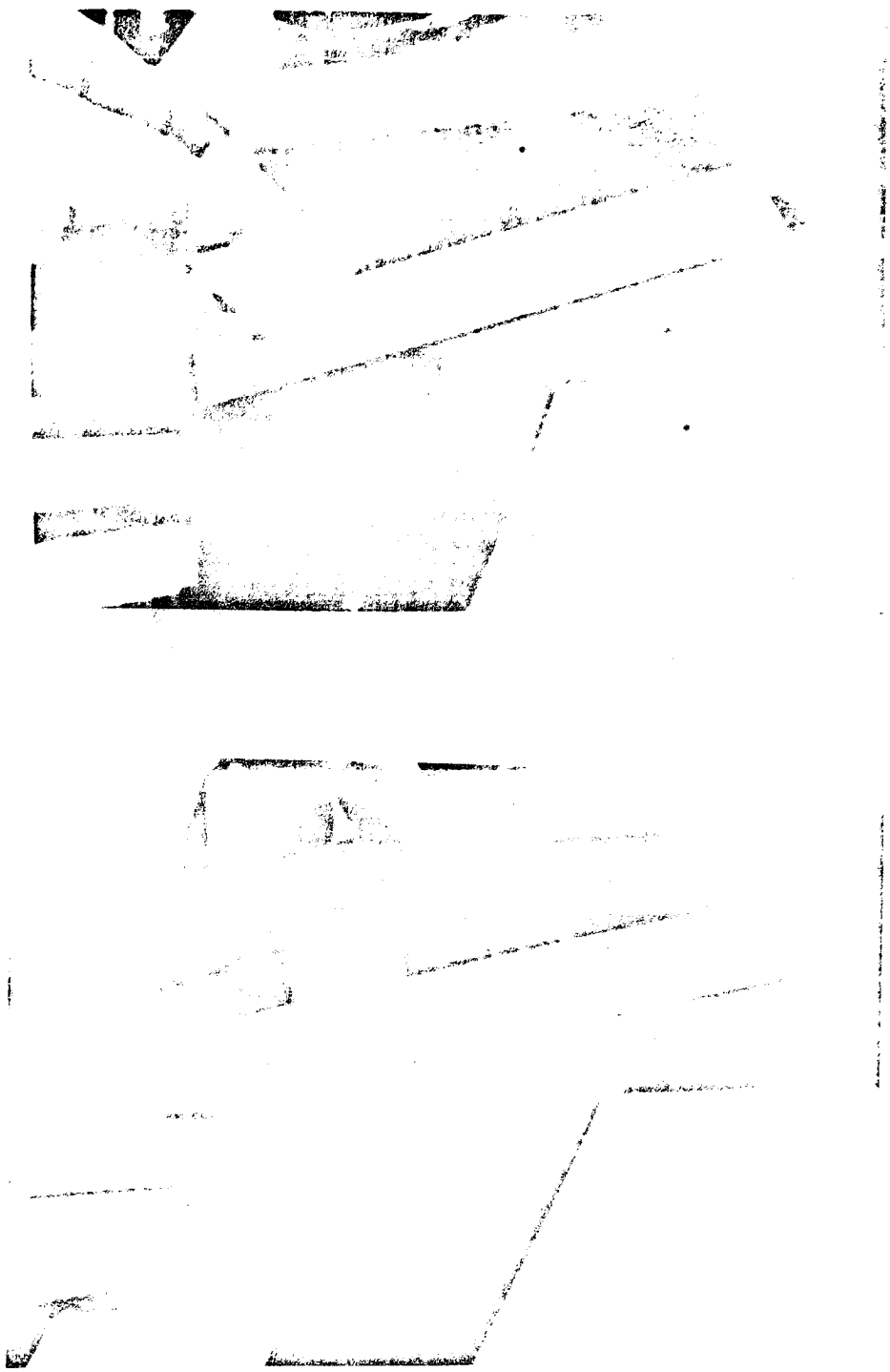


Figure 17

Use of the streaker device for quantitative applications of sample to the chromatogram

11. The streaker was placed on the chromatogram 20 mm from the bottom edge in one of the eight columns reserved for the run and its replicate. This was repeated for the other concentrations of hydroquinone.

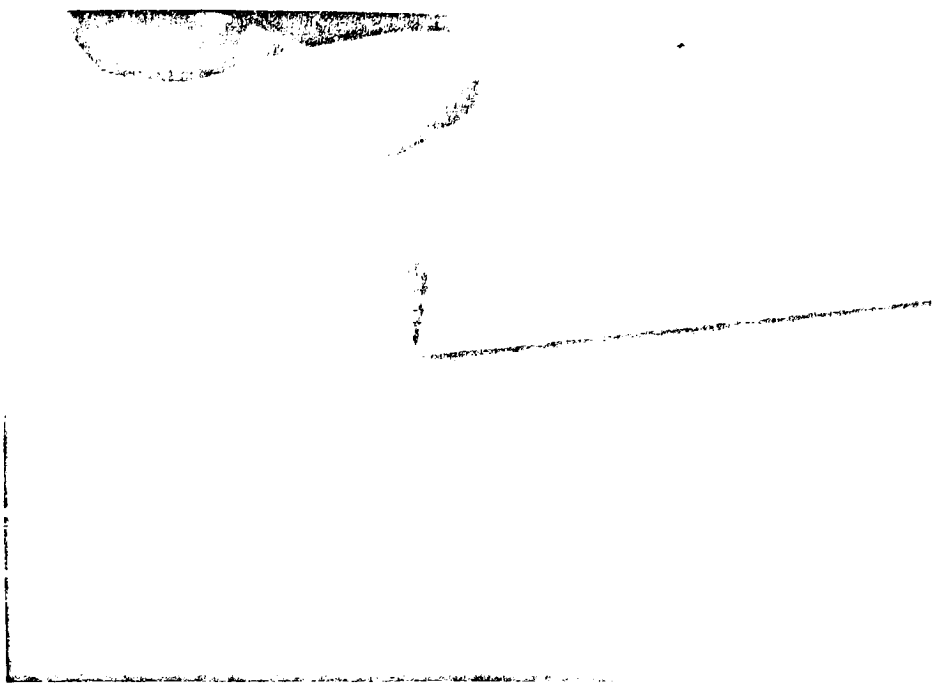
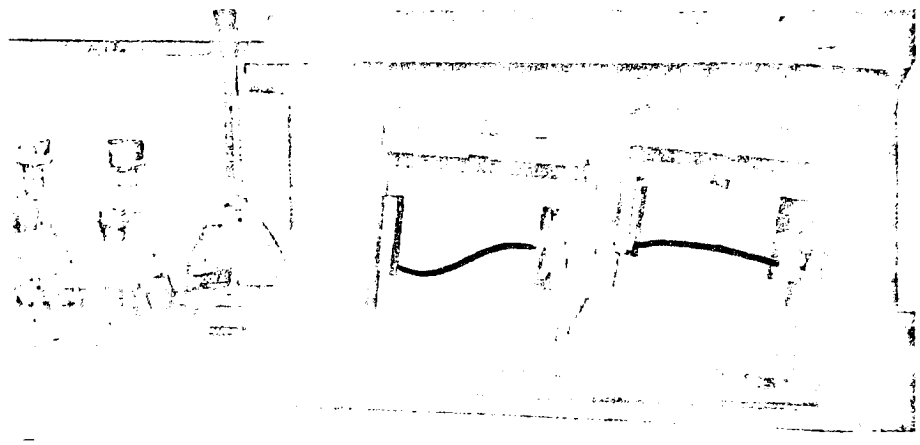


Figure 18

Application of streaker sample to the chromatogram

12. The silica gel chromatogram, being inactive with the eight spotted samples, was placed in one of the chamber plates. Another chromatogram with eight other spotted samples at the higher concentration of sodium sulfite but the same pH level, was placed in the other chamber plate apparatus. Samples spotted on the chromatogram were allowed to dry before elution.

13. An n-butanol:acetic acid:water (80:5:15 parts by volume) solvent system was used with the silica gel chromatogram and was eluted for five and a half hours with 150 ml of the solvent system placed in the reservoir. During the course of elution, the temperature was between 72 and 75° F. and the relative humidity was between 30 and 40%. At times an uneven solvent front was observed. It was theorized that temperature and humidity gradients were responsible for the uneven solvent front.



a) uneven solvent front b) even solvent front

Figure 19

To remedy this, the chromatogram was activated for

fifteen minutes in a 100° C. dry oven and allowed to stay over night on an open shelf to rejoin equilibrium with the atmosphere uniformly over the chromatogram.

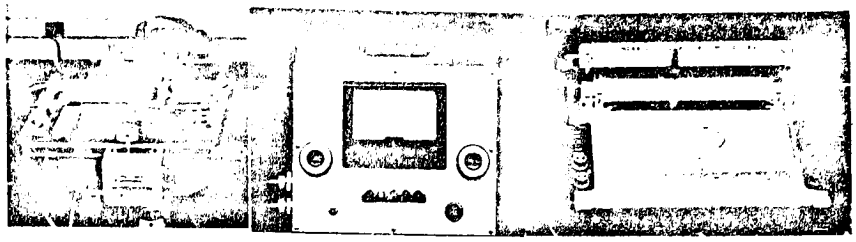
14. After elution, the chromatogram was dried in a low temperature oven.

15. Visualization of the hydroquinone and species formed in developer solution was done by spraying the chromatogram with .1N ammoniacal silver nitrate with an atomizer in a repeatable manner. The amount of silver nitrate used was enough to saturate the chromatogram.

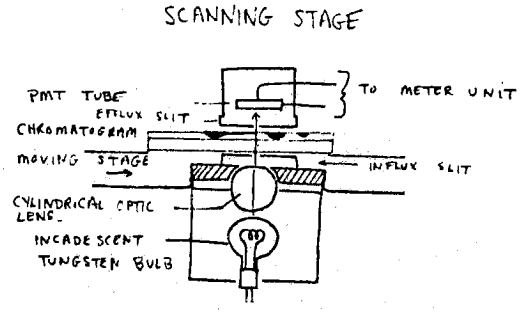
16. After visualization, the chromatogram was dried and the visualization reagent was disposed of by flushing it down a sink drain. Visualization was done one hour before the densitometric scanning.

17. Quantitization of the hydroquinone eluted on the chromatogram was achieved by measuring the maximum density of the hydroquinone reduced by the ammoniacal silver nitrate.

18. This maximum density was obtained by using a Photovolt TLC densitometer, model #520.



a) b) c)



a)

Figure 20

Photovolt TLC densitometer, model 520, with a) scanning stage, b) metric unit and c) recording pen.

The procedure for use of this densitometer was followed according to the manufacturer's operating instructions. The following steps in addition, were followed:

a. Before tracing the chromatogram, the scanning stage, meter and recording pen units were warmed up for twenty minutes.

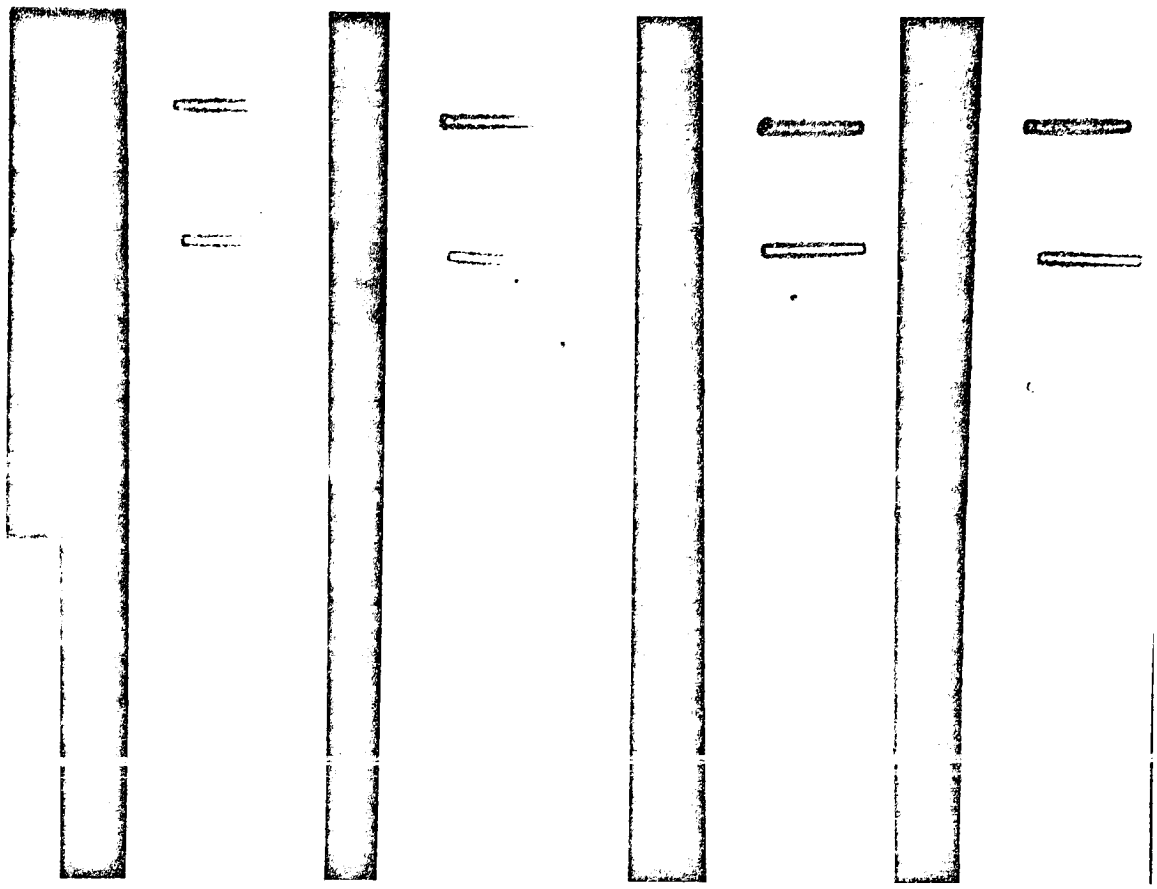
b. Lights in the room were turned off, since they affected density readings.

c. A Kodak #2 step tablet was traced to observe how the unit was performing.

d. On a point midway between the sample deposition line and the bottom edge of the chromatogram, the densitometer meter unit and recording pen were adjusted to zero by controlling the intensity of the tungsten lamp and line voltage controls to the metering and recording pen unit.

e. The densitometer meter unit and recording pen unit were adjusted to infinite density after covering the photo tube in the scanning stage with the opaque cover slit.

f. This was done before tracing every one of the sixteen runs done per day. Usually the runs were done the day before the scanning and the visualization was done one hour before scanning. This was done due to the problem of the browning effect.

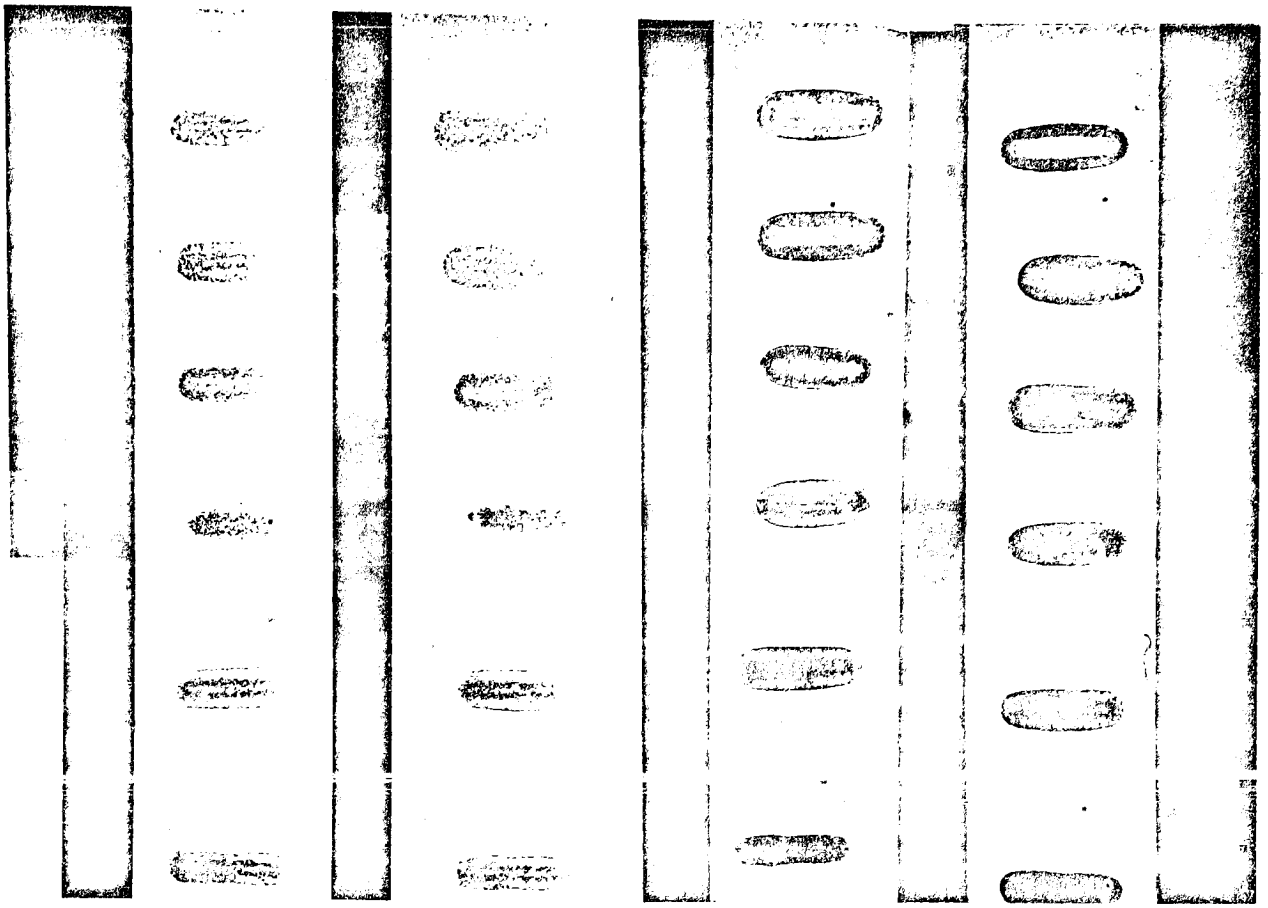


a)

Figure 21

Difference in visual appearance of chromatogram after visualization

a) 1 hour after visualization, b) 2¼ hours after visualization



b)

Figure 21

19. The paper used for the recording pen was made for the percent absorption mode of operation of the TLC densitometer unit. Table III was constructed to convert absorption units to density units. This was done by lining up the density pattern on the linear graph paper and reading off what density corresponded to the percent absorption.

20. For each specie or R_f value of hydroquinone, a denmaxima, Dm was obtained from the densitometric trace by referring through Table III for the correct density value. This was best illustrated by Figure 17 which showed a series of samples 1-8 that were deposited, eluted and visualized in the manner described in the procedure section. Each run was densitometrically scanned and where there was a peak, a density maxima was noted by drawing a bar over that peak.

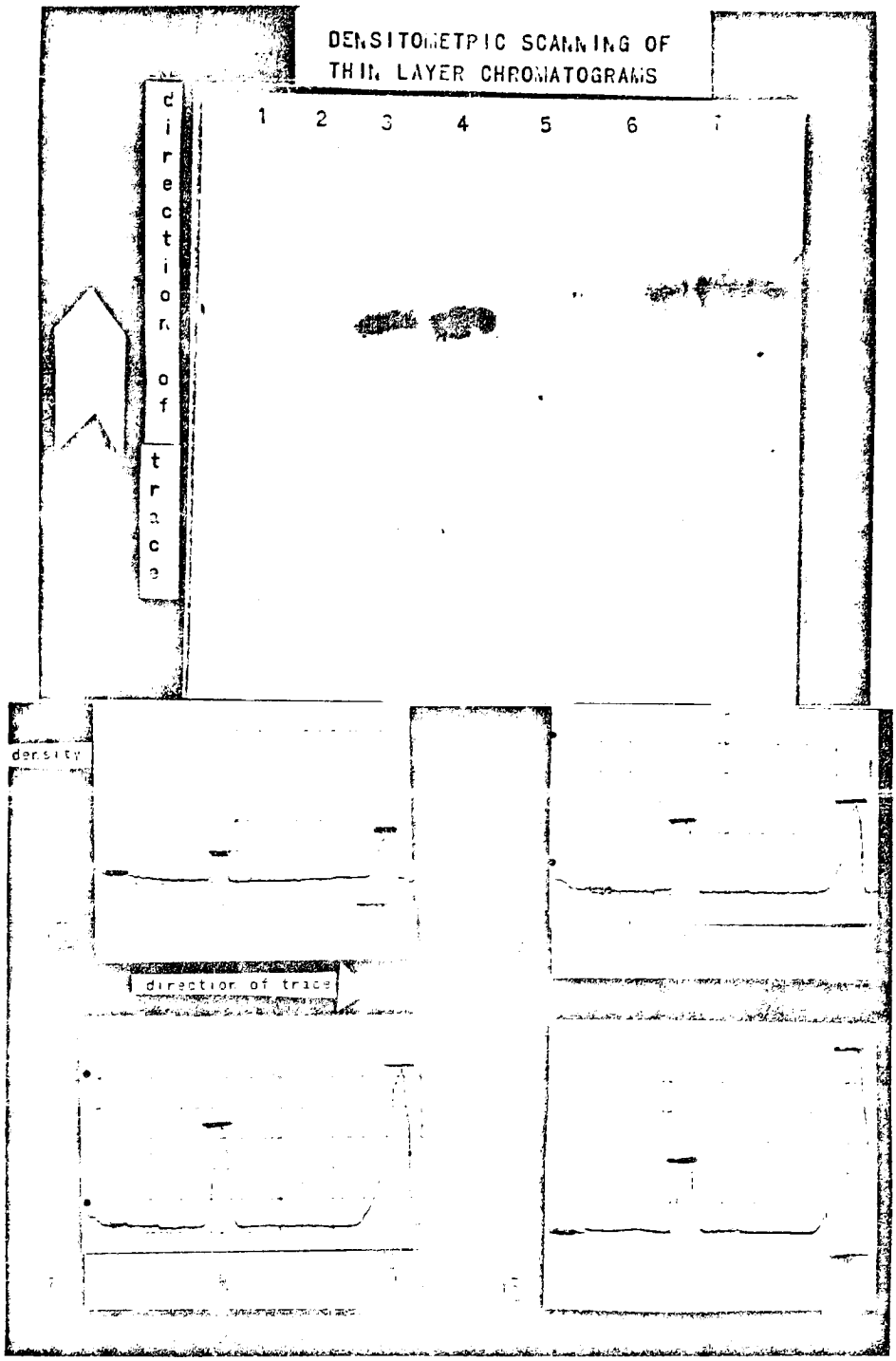


Figure 22

Illustration of how density maxima $D_m(1)$, $D_m(2)$ and $D_m(3)$ were obtained for species $R_f(1)$, $R_f(2)$ and $R_f(3)$ respectively.

Table III

Conversion of Absorption to Density on 520 TLC Platen

A	D	A	D	A	D	A	D	A	D
.00	.00								
.01	.012	.21	.262	.41	.510	.61	.762	.81	1.01
.02	.025	.22	.275	.42	.525	.62	.778	.82	1.02
.03	.040	.23	.288	.43	.540	.63	.790	.83	1.04
.04	.050	.24	.300	.44	.550	.64	.800	.84	1.05
.05	.062	.25	.312	.45	.565	.65	.812	.85	1.07
.06	.076	.26	.325	.46	.575	.66	.825	.86	1.08
.07	.088	.27	.338	.47	.588	.67	.838	.87	1.10
.08	.10	.28	.350	.48	.600	.68	.850	.88	1.12
.09	.11	.29	.362	.49	.612	.69	.862	.89	1.14
.10	.125	.30	.375	.50	.625	.70	.875	.90	1.16
.11	.140	.31	.388	.51	.638	.71	.888	.91	1.18
.12	.150	.32	.395	.52	.650	.72	.895	.92	1.20
.13	.160	.33	.410	.53	.660	.73	.910	.93	1.25
.14	.175	.34	.420	.54	.675	.74	.920	.94	1.35
.15	.188	.35	.435	.55	.688	.75	.935	.95	1.50
.16	.200	.36	.445	.56	.700	.76	.945	.96	
.17	.210	.37	.460	.57	.712	.77	.960	.97	1.75
.18	.225	.38	.470	.58	.725	.78	.970	.98	2.00
.19	.240	.39	.485	.59	.738	.79	.985	.99	
.20	.250	.40	.500	.60	.750	.80	1.00	1.00	∞

A= Absorption; D= Density.

21. The log density of Dm(1), Dm(2) and Dm(3) were tabulated for runs 1-48 and plotted as a function of log concentration of the hydroquinone developing agent, prepared in solution at a fixed sodium sulfite concentration and pH level. This required six graphs to do for the 48 runs.

Observations

1. For each corresponding specie of hydroquinone there was an R_f value.
2. At lower pH levels, the $R_{f(1)}$ specie did not appear.
3. The appearance of the chromatogram changed with time after the visualization. At higher concentrations of hydroquinone deposited on the chromatogram, insufficient application of the visualization reagent resulted in densities that were not significantly darker (visually) than the lower concentrations of hydroquinone.

Data and Conclusions

Table IV

Density maxima Dm(1), Dm(2) and Dm(3) for runs 1-48

run	Dm(1)	$\overline{Dm(1)}$	$\Delta Dm(1)$	Dm(2)	$\overline{Dm(2)}$	$\Delta Dm(2)$	Dm(3)	$\overline{Dm(3)}$	$\Delta Dm(3)$
1	.03			.11			.18		
5	.05	.04	.02	.18	.15	.07	.21	.20	.03
2	.04			.25			.33		
6	.06	.05	.02	.30	.28	.05	.38	.36	.05
3	.02			.33			.68		
7	.03	.03	.01	.44	.37	.07	.65	.67	.03
4	.03			.33			.78		
8	.05	.04	.02	.30	.32	.03	.78	.78	.00
9	.06			.04			.25		
13	.09	.08	.03	.07	.06	.03	.28	.27	.03
10	.08			.05			.39		
14	.12	.10	.04	.18	.12	.13	.36	.38	.03
11	.03			.10			.55		
15	.06	.05	.03	.09	.10	.01	.50	.53	.05
12	.05			.25			.76		
16	.09	.07	.04	.28	.27	.03	.81	.79	.05
17	.05			.29			.23		
21	.04	.05	.01	.24	.27	.03	.21	.22	.02
18	.06			.39			.36		
22	.03	.05	.03	.34	.37	.04	.35	.36	.01
19	.05			.66			.58		
23	.04	.05	.01	.63	.65	.03	.53	.56	.05
20	1.50			1.02			.63		
24	.57	1.04	.93	.80	.91	.22	.57	.60	.06
25	.34			.14			.31		
29	.31	.33	.03	.13	.14	.01	.31	.31	.00
26	.36			.15			.40		
30	.36	.36	.00	.16	.16	.01	.41	.41	.00
27	.57			.30			.78		
31	.53	.55	.04	.30	.30	.00	.59	.67	.19

Table IV (cont'd)

run	Dm(1)	$\overline{Dm}$(1)	Δ Dm(1)	Dm(2)	$\overline{Dm}$(2)	Δ Dm(2)	Dm(3)	$\overline{Dm}$(3)	Δ Dm(3)
28	.66			.55			.92		
32	.53	.60	.13	.35	.45	.20	.57	.75	.35
33	.68			.51			.10		
37	1.35	1.02	.67	.81	.66	.30	.63	.36	.53
34	.66			.79			.20		
38	.86	.76	.20	.83	.81	.04	.15	.18	.05
35	1.50			1.04			.76		
39	1.20	1.35	.30	.97	1.01	.07	.57	.65	.11
36	1.60			1.14			.65		
40	1.05	1.33	.55	.73	.94	.41	.63	.64	.02
41	.36			.20			.24		
45	.50	.42	.14	.10	.30	.10	.08	.16	.16
42	.74			.53			.10		
46	.46	.75	.32	.35	.88	.18	.35	.23	.25
43	.81			1.16			.80		
47	.59	.70	.22	1.14	1.15	.02	.68	.74	.12
44	.86			1.75			.86		
48	.88	.87	.02	1.35	1.55	.40	.78	.82	.08

Δ Dm is referred to as the range, when plotted on graphs 1-6.

Conclusion

1. A regressional analysis should be done for $D\bar{m}(3)$ vs. the log concentration of hydroquinone at fixed levels of sodium sulfite concentration and pH but time did not permit this to be done.
2. In runs 1-48, the log Dm(3) is an increasing function of log hydroquinone concentration over all sodium sulfite concentrations and pH levels. It appears that with the experimental estimate of error, $\Delta Dm(3)$, that it is a linear relationship.
3. At higher concentrations of sodium sulfite and pH, $D\bar{m}(2)$ and $D\bar{m}(3)$ are increasing with increasing hydroquinone concentrations. This should indicate that the hydroquinone monosulfonate formed at higher concentrations of sodium sulfite and pH. The author would think that the concentration of hydroquinone would decrease. The hydroquinone concentration as indicated by $D\bar{m}(3)$ should then decrease, but this has not been proven.
4. It was observed that the amount of visualization reagent sprayed on a chromatogram with an atomizer, was a very inaccurate, non-uniform manner of application.

A device was built to uniformly deposit the visualization reagent. It was similar to a rotating postage stamp moistener. The rotating pyrex cylinder was sand-blasted to eliminate the lipophillic

properties of the glass. The ammoniacal silver nitrate was picked up by the rotating cylinder and a uniform amount of the reagent was applied to the traveling chromatogram by a meniscus transfer. However, due to engineering parameters, the quantity of silver nitrate transferred was not sufficient to produce higher densities at higher concentrations of hydroquinone.

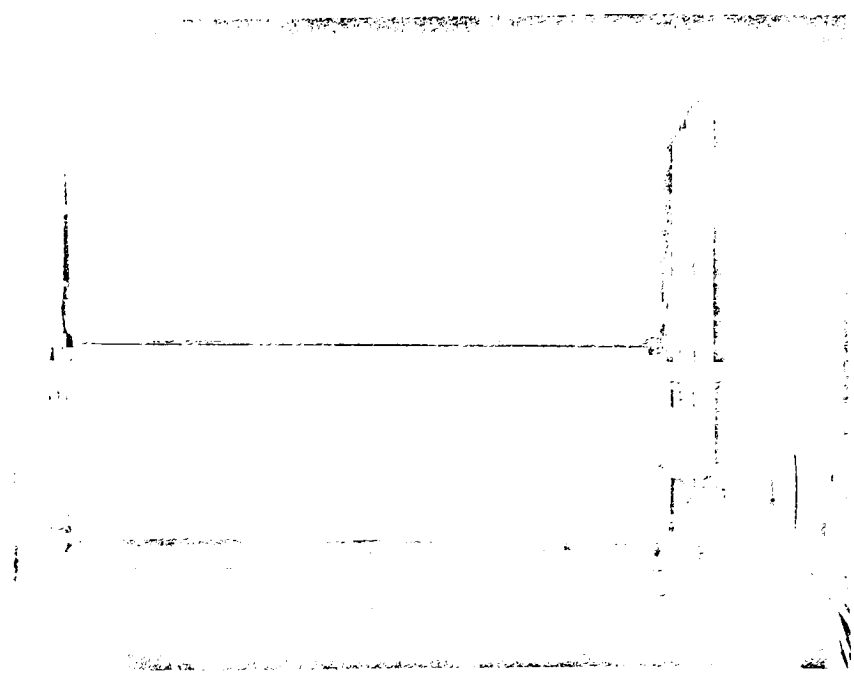


Figure 22

5. By estimation of the amount of silver (formed by the reduction of the 1N ammoniacal silver nitrate solution by the hydroquinone) through transmission

densitometry, it should have been possible to estimate the amount of hydroquinone that was eluted on the chromatogram.

6. Because of the following problems, which were not standardized or controlled, non-random experimental error was introduced.
   a. Repeatability of densitometric results using the Photovolt TLC 520 densitometer.
   b. Application of visualization reagent.
   c. Instability of the chromatogram after visualization (see figure 19).
7. Because of those non-random factors that could affect the experimental error, the procedure describing quantitative analysis of the hydroquinone developing agent was at best a semi-quantitative analysis. The author will propose methods of reducing the experimental error to make TLC a more accurate quantitative analysis in Appendix A.
8. One advantage of using TLC for quantitative analysis of a photographic developing agent hydroquinone was that it could determine the quantity of oxidized species of hydroquinone formed. There are disadvantages of quantitative TLC when compared to the conventional chemical analysis of photographic developing agent.[22] These conventional procedures require

---

[22]Chemical Control Procedures for Black and White Film Processing, Motion Picture Film Department, Nov., 1962, Eastman Kodak Co., Rochester, New York.

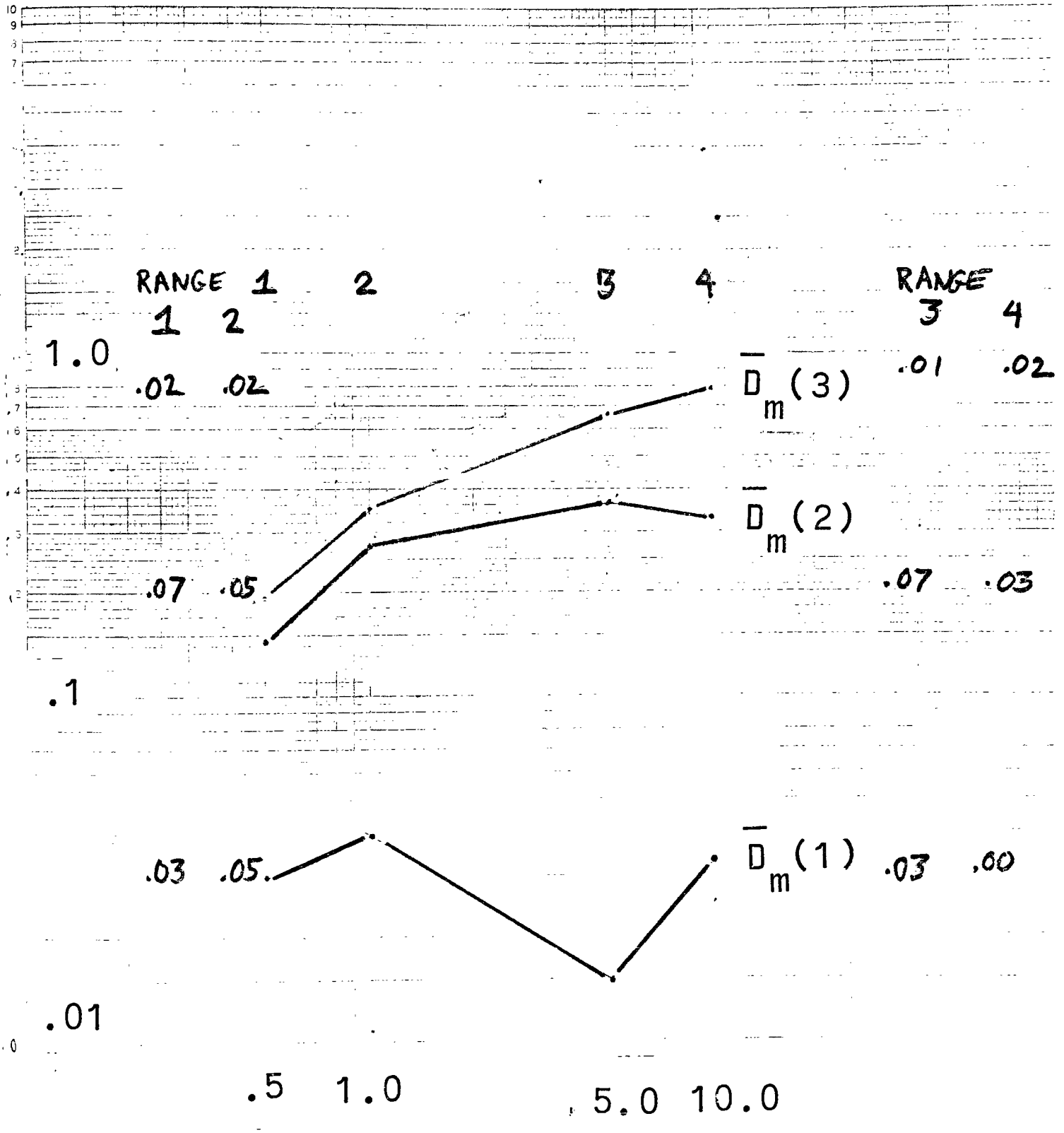
minutes to perform the quantitative analysis. However, procedures are restricted in the sense that they can be applied only to the specific purpose they were designed for. Often, they can not be applied to other developer solutions containing different developing agents. This procedure has been modified to analysis of other developing agents. However, these procedures are of a proprietary nature and are not readily available on request.

9. Additional solvent systems and chromatographic substrates will be proposed in Appendix B for TLC with faster elution conditions and better separation of developing agents.

Log density vs. concentration of H_2Q

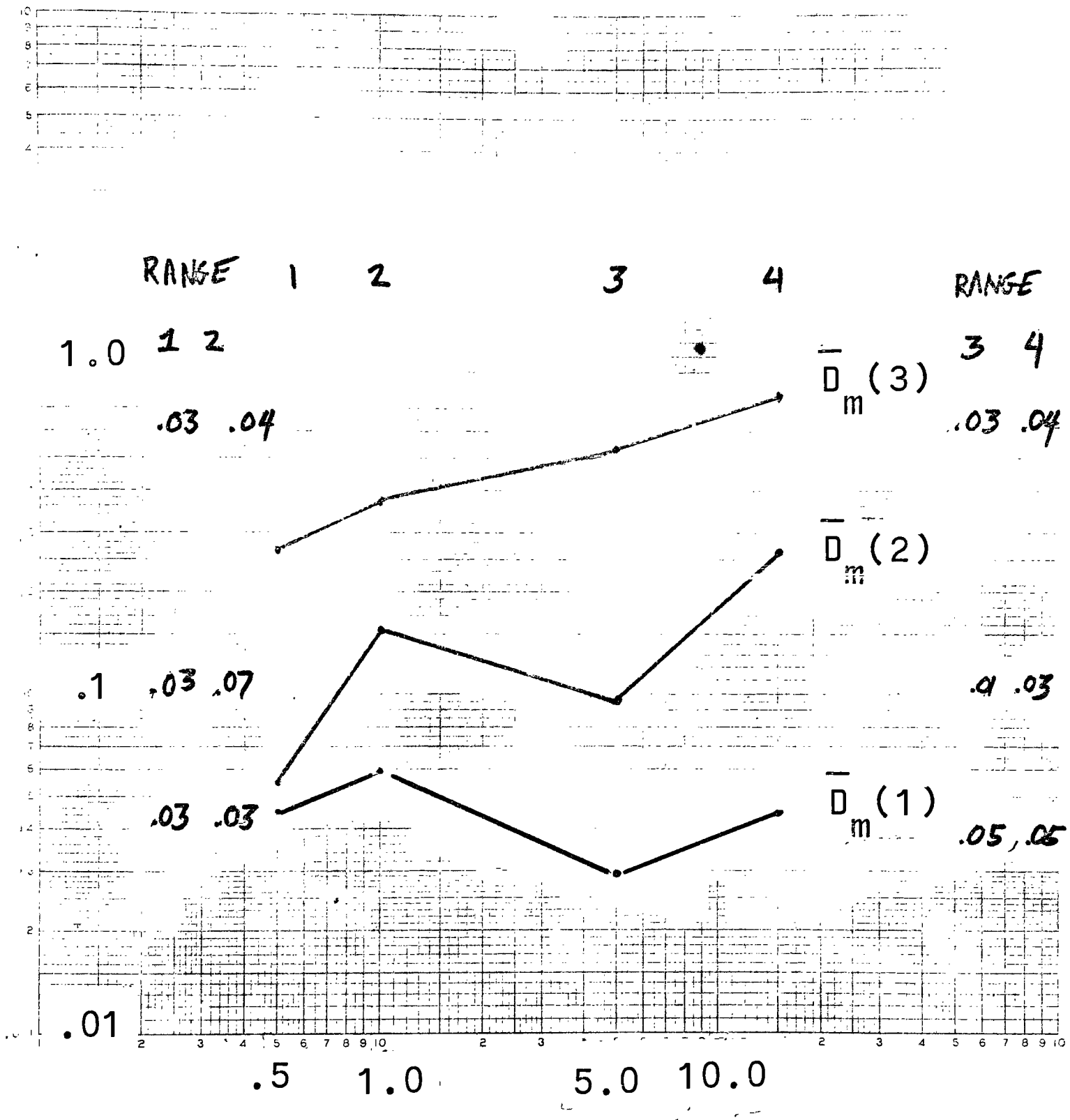
run 1-8: pH= 9.0, sodium sulfite= 10.0 g/l.

Graph I



run 9-16: pH= 9.0, sodium sulfite= 100.0 g/l

Graph II

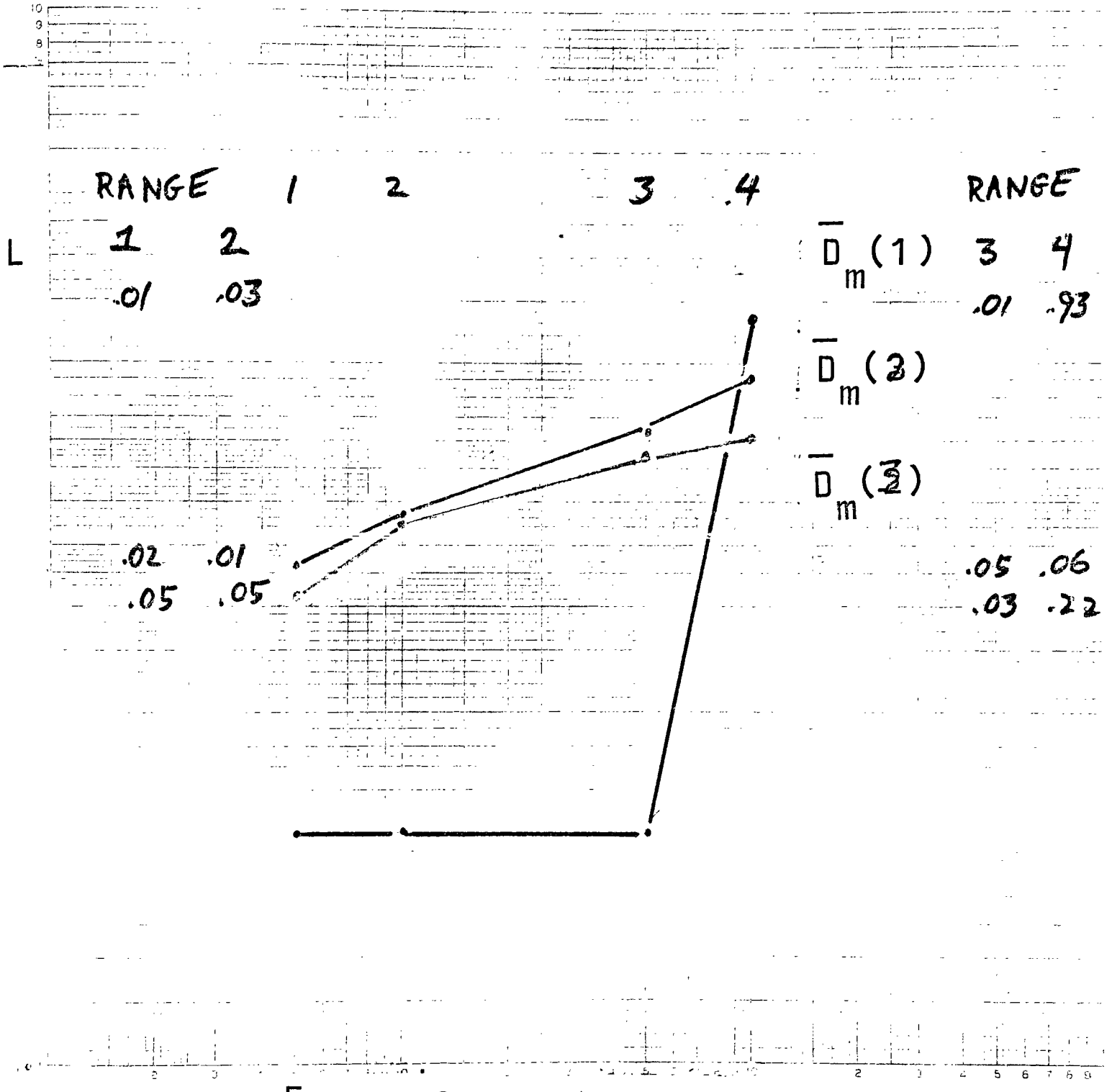


Full Logarithmic, 3 × 3 Cycles

Log density vs. concentration of H_2Q

run 17-24: pH= 11.0, sodium sulfite= 10.0 g/l

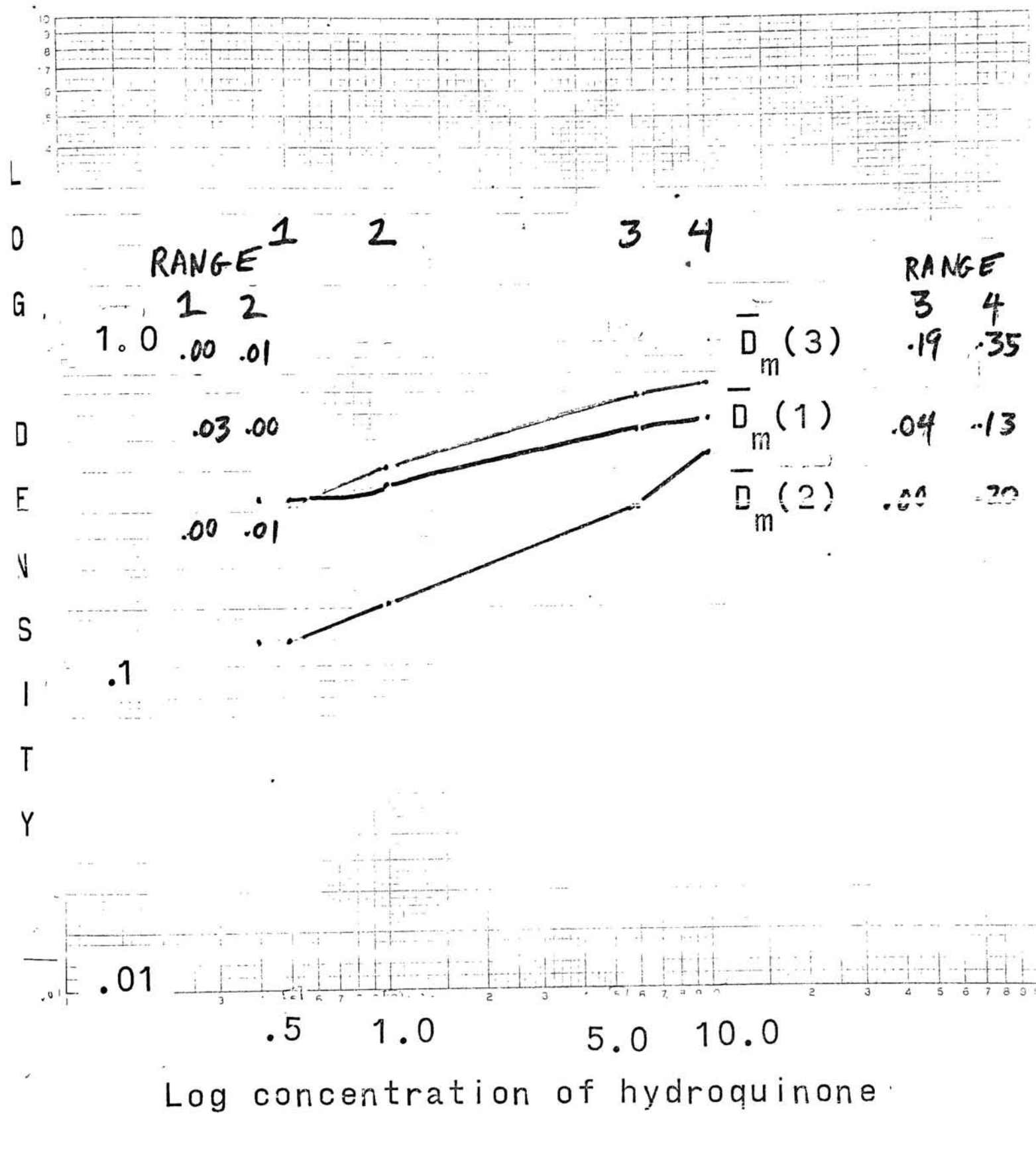
Graph III



Log density vs. concentration of H_2Q

run 25-32: pH= 11.0, sodium sulfite= 100.0 g/l

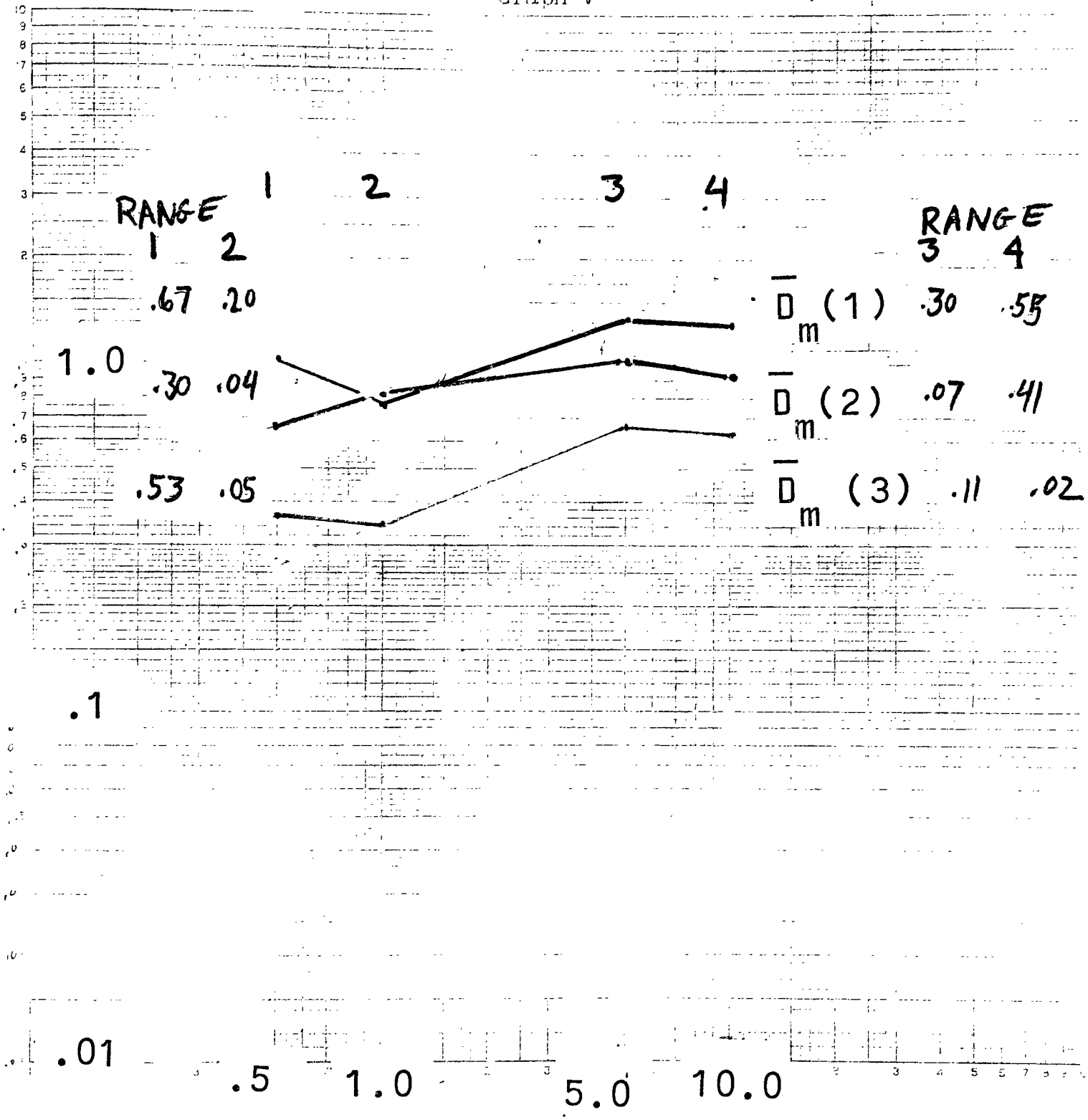
Graph IV



Log density vs. concentration of H_2Q

run 33-40: pH= 13.0, sodium sulfite= 10.0 g/l

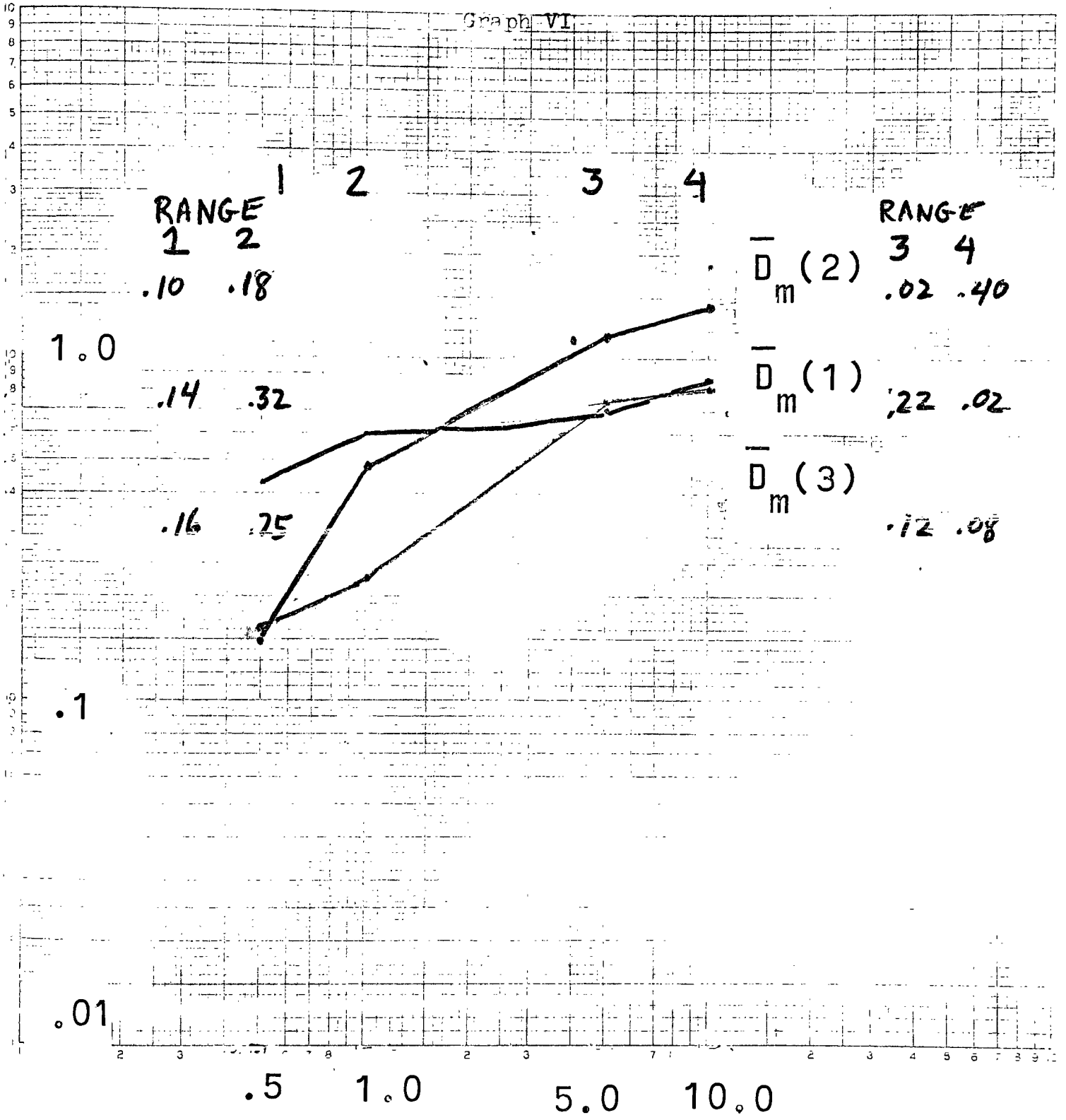
Graph V



Log concentration of hydroquinone

Log density vs. concentration of H_2Q

run 41-48: pH= 13.0, sodium sulfite= 100.0 g/l



BIBLIOGRAPHY

BIBLIOGRAPHY

Abbott, D. and Andrews, R.S., An Introduction to Chromotography, Boston, Houghton Mifflin Company, 1965.

Babbit, James, Chromatographic Analysis, New York, Reinhold Book Corporation, 1968.

Blaedel and Meloche, Elementary Quantitative Analysis, 2nd edition, New York, Harper and Row, 1953.

Distillation Products Industries, "A File of Analytical Procedures for use with the Eastman Chromagram System," publication z7-5, Rochester, New York, November, 1965.

Eastman Kodak Company, "Chemical Control Procedures for Black and White Film Processing," Motion Picture Film Department, Rochester, New York, November, 1962.

Eastman Kodak Company, Eastman Organic Chemical Catalogue #46, pp. 391-392, Rochester, New York, 1971.

Eastman Kodak Company, "Eastman TLC Visualization Reagents and Chromatographic Solvents," Publication #JJ-5, p. 8, Rochester, New York, July, 1971.

Eggers, J., "Photo. u. Wiss.," 10, 40, 1961.

James, T.H., "Analysis of Developing Agents by Paper Chromatography," Photographic Science and Engineering, Vol. 3, 1959.

Karnsk, F.W., "Thin Layer Chromatography," Research/Development, 4, 1973.

Lapp, Reiner, "Brinkman Bibliography for TLC," Westbury, New York, Brinkman Instruments, Inc.

Mariani, B. and Martinelli, P., "Sci. Ind. Photog.," p. 276, Series II, No. I, 1956.

Mees, C.E.K. and James, T.H., The Theory of the Photographic Process, 3rd edition, Macmillan Company, 1969.

Pannel, J.A. and LuValle, J.E., "Anal. Chem.," Vol. 25, p. 1566, 1953.

Randerath, Kurt, Thin Layer Chromatography, 2nd edition, pp. 70-79, New York, Academic Press, 1969.

Russel, G., Chemical Analysis in Photography, Chapter 8, New York, Focal Press, Ltd., 1965.

Stahl, Egon, Thin Layer Chromatography, 2nd edition, New York, Springer Verlog, Inc., 1969.

Todd, H.N. and Zakia, R.D., Photographic Sensitometry, New York, Morgan and Morgan Inc., 1969.

APPENDICES

APPENDIX A

Propose Methods for Improving Quantitative TLC

A variety of methods have been used to quanticize the amount of substance on a thin layer chromatogram. In the past, quantitative TLC had limited accuracy (10 to 20 per cent) and was time consuming with hand operated methods.[23] Recently, spectrodensitometric instrumentation has been made that makes rapid and accurate determinations on the chromatogram. With this later day sophisticated instrumentation, many of the variables that lead to inaccurate results were eliminated compared to the earlier Photovolt TLC 520 densitometer. The Photovolt TLC 520 densitometer did not contain any of the automated features of the new breed of thin layer chromatogram scanners.

---

[23]Kurt Randerath, "Thin Layer Chromatography," pp. 70-79.

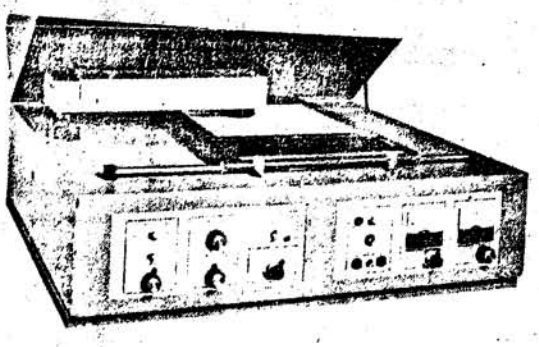


Figure 24[24]

5D-2000 Chromatogram Analyzer (Schoeffel Instrument Corp.)

The 5D-2000 chromatogram unit can measure transmission, reflectance, flouresence and adsorption. This unit uses a double beam ratio technique that eliminates many of the troubles encountered with the Photovolt TLC 520 unit. Figure 24 shows the schematic diagram of the 5D-2000 unit.

---

[24]Thin Layer Chromatography, Research/Development, op. cit., p. 38.

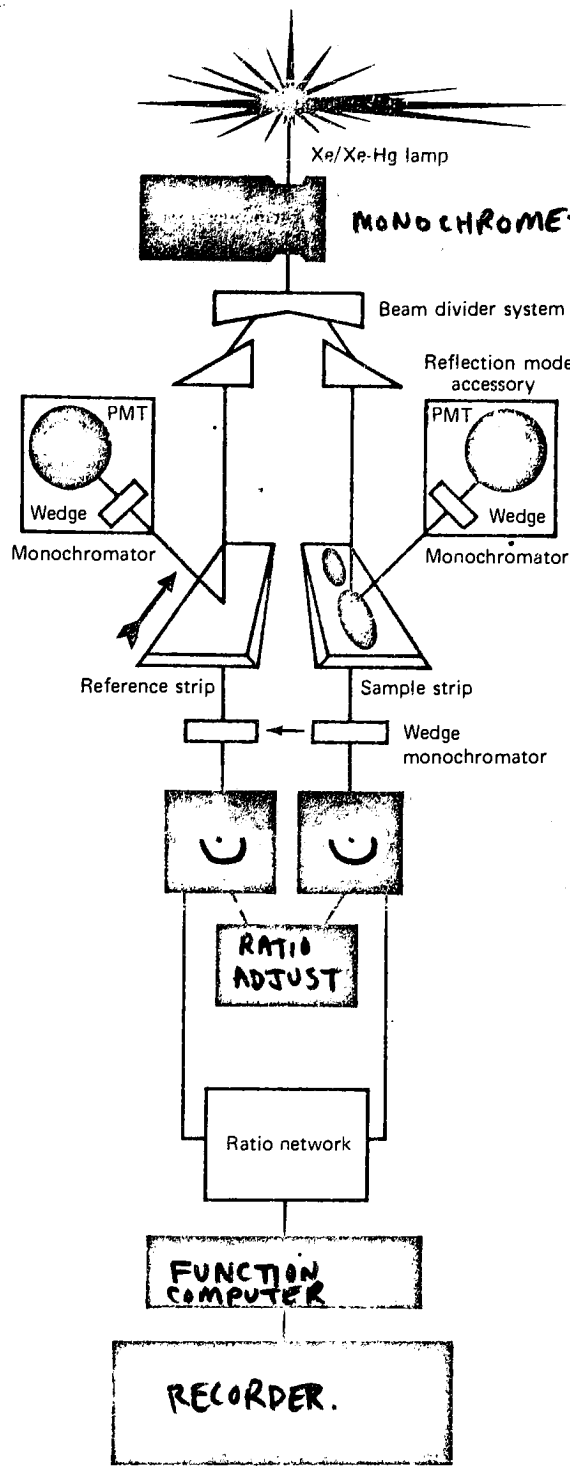


Figure 25[25]

Schematic diagram of a modern split/reference beam TLC scanner

[25] Ibid.

Photographic developing agents may not show any short wavelength or visible absorption. In this case, the developing agent may have to be visualized. The application of the visualization reagent would be critical if absorption of the visualized substance was to be used for quantitization. For this reason, a device was constructed to uniformly apply an accurate amount of visualization reagent (see figure 23). Dr. Carroll was responsible for the suggestion and the author of this thesis believed that such a device has considerable merit over hand application of the visualization reagent by a sprayer bottle. Unfortunately, some engineering parameters were not resolved to incorporate it into the factorial experiment.

Dr. Carroll also suggested the x-ray flouresence technique Kodak used for their quantitative determinations of silver. However, such an apparatus is costly and difficult to obtain.

Dr. Francis suggested two additional alternatives. The first one was to do a potentiometric titration of the silver formed when the hydroquinone was reduced by the .1N ammoniacal silver nitrate by scraping the blackened areas off of the chromatogram. The other method, suggested by Dr. Francis, would be to scrape off the area of the chromatogram, which was known to contain the hydroquinone, and perform a sulfato cerrate titration to an indicated end

point with a Ferroin indicator.[26]

---

[26]Chemical Control Procedures for Black and White Film Processing, Procedure 407B.

APPENDIX B

Solvent systems and substrates may exist that would have shorter elution times and promote sharper separation of photographic developing agents. Dr. Harry Baden, of the Eastman Kodak Company Photo Technology Division in Rochester, New York, suggested the following solvent systems to be used with activated silica gel chromatograms.

solvent system:

1. benzene:methanol:acetic acid (90:36:36 v/v)
2. ethyl acetate:acetone:acetic acid:water (100:60:20:20 v/v)
3. chloroform:ethyl acetate:acetic acid (140:40:20 v/v)
4. benzene:methanol:acetic acid (180:32:32 v/v)

Stahl listed solvent systems and chromatogram substrates used to separate phenol, pyrogallol and resarcinol.

1. dioxane:benzene:acetic acid (25:90:4)
2. methanol:benzene:acetic acid (8:45:4)

To what extent the solvent systems would separate the developing agents and the time required for adequate elution is not known. Work remains to be done in this area.